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Local Day

Possible tasks and new areas for blood banks

1A-01-01

PATHOGEN REDUCTION-WHAT ARE THE ADVANTAGES, WHAT ARE THE PROBLEMS WHAT ARE THE COSTS?

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Blood transfusions have never been safer. However due to the fact that it is a transfusion of allogeneic cells from one person to another there will always be a risk.
Different deferrals of donors due to risk behaviour have reduced pathogen transfers.
The introduction of "double cleansing" combined with a sample pouch has reduced the risk of skin contamination from the donor to the collection bag.
Increased testing of the donors has also reduced pathogen transfer. Testing of pathogen is always a reactive way of avoiding transmission. First you need to know what pathogens are involved and then you must have a test. A different way is to be proactive and have some sort of pathogen reduction technique.
Pathogen reduction techniques (PRT) have been used for many years in the production of plasma derivates mainly to omit transfer of viruses. Always with some loss of the substance you would like to have left.
In western countries bacterial contamination of transfusions products is more frequent than viral infections. Bacteria are a main problem in platelet preparation due to the storage conditions. The infection prevalence ranges between 1:1000 and 1:5000 per platelet concentrate unit, while the risk in red cells is much lower. Bacterial screening, mandatory in many countries in Europe and the USA, has decreased the risk. As always sampling can be tricky. Is the sample representative? How long should the sample be cultured before release etc. There are studies indicating that even if the bacterial screening was reactive it did not stop the transfusion due to different reasons.
We always have emerging pathogens both new and those that are re-emerging. PRT would be a more convenient way to deal with that problem. All PRT comes with a loss of what you would like to preserve. The loss of platelets during pathogen reduction can be dealt with by increasing the amount you start with if these platelets are hemostatically equivalent to control platelets. Most studies have shown that but a few have shown otherwise. When dealing with plasma PRT, some countries do not allow specific PRT to some patients. At the moment there are no commercial available PRT for red cells or whole blood.
The amount of patients dying of acute transfusion transmitted infectious diseases are few but many of those getting bacteria contaminated platelets are on antibiotics or could be treated in time which could explain that.
The use on PRT is a proactive way to deal with pathogens. The problem is that you can't prevent transfers of every sort of pathogen and you get at least some loss. However that can be taken care of in your logistics. The cost will increase but that can in some way be taken care of with lower outdating (platelets and quarantine plasma).
PRT can also be away to calm the public demand for "safe" blood transfusions, but one must have in mind the loss during PRT and take precautions.

1A-01-02

HPA-1A ANTIBODY SCREENING IN PREGNANCY: IS IT POSSIBLE AND WHAT ARE THE POTENTIAL OUTCOMES?

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Foetal and neonatal alloimmune thrombocytopenia (FNAIT) is a rare but potentially very serious bleeding condition affecting the foetus/newborn. The vast majority (>80%) of FNAIT cases among Caucasians, and the most serious one's, are caused by maternal antibodies to the Human Platelet Antigen 1a (HPA-1a). Anti-HPA-1a of IgG class can traverse the placenta and cause foetal thrombocytopenia. Thus, FNAIT can be considered as the platelet counterpart to haemolytic disease of the foetus and new-born (HDFN). The clinical consequences of FNAIT spans a continuum from no symptoms to petechiae, mucosal bleeding, haematomas, retinal bleeding and intracranial haemorrhage (ICH), which may lead to intrauterine death or lifelong disability. The incidence of FNAIT-associated ICH has been estimated to be around 1 in 10,000 newborns, which translates to approximately 1,000 yearly FNAIT-associated cases of ICH in Europe and North America.
Most cases of FNAIT are discovered when a child is born at term with petechiae or other signs of bleeding in the absence of any other condition known to be associated with neonatal thrombocytopenia. Without a screening program for HPA-1a negative pregnant women, primary prevention is not an option. Secondary prevention is only possible in those cases where a woman has previously given birth to a child with FNAIT. Due to insufficient data from randomized controlled trials there is currently no international consensus regarding the optimal antenatal management of women with an obstetric history of FNAIT. In lack of an approved treatment, most centres use high-dose intravenous immunoglobulin (IVIG) off-label for treatment of pregnant women who previously have given birth to a child with FNAIT, in order to mitigate the risk of severe bleeding in the foetus/newborn.
For many reasons, FNAIT has not been considered for prophylactic efforts similar to those that have been used for the last four decades for the prevention of HDFN, caused by antibodies against RhD: First, HPA-1a typing kits are not well suited to the workflow in immunohaematology laboratories; secondly, the costs associated with HPA-1a typing of all pregnant women has been a hindrance; thirdly, there is no international consensus regarding the optimal antenatal management of HPA-1a-immunized women identified in a screening programme; fourthly, it has generally been believed, that immunization against HPA-1a mostly takes place during the first incompatible pregnancy, and finally, there is yet no medicinal product available that can prevent HPA-1a-immunization in the same way as anti-D can prevent RhD-immunization.
Implementation of HPA-1a typing of all pregnant women has been discussed by the health authorities in several European countries. It is questionable to what extent general HPA-1a typing fulfils WHO's criteria for screening programs laid down by Wilson and Jungner in 1968, and so far, general HPA-1a typing has not yet been implemented in any countries. The abovementioned five hindrances for FNAIT screening and the Wilson and Jungner criteria will be discussed in the light of recent advances within FNAIT research.

1A-01-03

ATMP: THE ROLE OF THE BLOOD BANK

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No abstract available

1A-01-04

PERSONALIZED TRANSFUSION MEDICINE

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Independent of the cause, massive hemorrhage is associated with high risk of coagulopathy and excess mortality, hereby being a major cause of potentially preventable deaths.
Until 2006, transfusion guidelines recommended that resuscitation of massive hemorrhage should occur in successive steps using crystalloids, colloids and packed red blood cells (pRBCs) in the early phase followed by plasma and platelet concentrate (PC) in the late phase. In 2007, this concept was however challenged by results from the US Military in Iraq demonstrating improved survival in patients receiving plasma (thawed fresh frozen plasma, FFP) together with pRBCs and PCs from the start of resuscitation, results that were soon confirmed in civilian trauma patients. Along with the introduction of *damage control surgery*, a concept prioritizing early control of the cause of bleeding by temporary, non-definitive means, *hemostatic control resuscitation* evolved as a concept prioritizing early control of the coagulopathy.
The introduction of hemostatic control resuscitation was followed by an emerging understanding of acute trauma induced coagulopathy (TIC), revealing different types of coagulopathy i.e. hypercoagulability, hypocoagulability including hypofibrinogenemia, endogenous anticoagulation and auto-heparinization, platelet dysfunction, hyperfibrinolysis and shock induced endotheliopathy (SHINE).
The finding that the clinical phenotype of coagulopathy reflected distinct pathophysiologic types of coagulopathy requiring different types of treatment, revealed an urgent need for adequate hemostatic monitoring i.e., a monitoring both capable of timely identification and classification of the coagulopathy and of guiding the hemostatic therapy. The viscoelastic hemostatic whole blood assays,

Thrombelastography (TEG®) or Rotation Thromboelastometry (ROTEM®) combined with whole blood platelet aggregation assays like e.g. Multiplate®, represent adequate hemostatic monitoring that in a timely manner identify and classify the different types of coagulopathy and at the same time guide therapy. The implementation of these assays have resulted in goal-directed hemostatic resuscitation through goal-directed administration of plasma, PC, cryoprecipitate, fibrinogen concentrate, factor concentrates, anti-fibrinolytics and protamine sulphate and have thus paved the way for personalized transfusion medicine. This approach has improved our understanding of coagulopathy, reduced morbidity and improved survival of massively hemorrhaging patients.

Use and future use of blood donor and transfusion databases

1B-02-01

PERSPECTIVES AND OPPORTUNITIES FOR THE DANISH BLOOD DONOR STUDY

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The Danish Blood Donor Study (DBDS) was initiated in 2010, and has since included 110,000 blood donors in a nation-wide prospective population study. In recent substudies, we have demonstrated that the platform not only facilitates studies of donor health, but also constitutes a highly cost-effective platform to answer general, health-related research questions. Indeed, access to a wide range of national health registers also available in other Nordic countries further augment the versatility of DBDS.

We are currently in the process of expanding DBDS with even more big data. Specifically, 20,000 donors have been selected for genotyping for 750,000 SNPs this year as part of a genome wide association initiative. Expansion of donor phenotypes is another strategy pursued to increase the available data. A simple, paper-based questionnaire was used early in the study; but the recent implementation of an electronic questionnaire has facilitated in-depth screening for several traits because it allows for a few screening questions to unfold a series of more detailed questions where relevant. In addition, new electronic means of establishing phenotype data, such as smartphone use, is planned. Likewise, the collection of specimens other than plasma and DNA is ongoing or planned; e.g., nasal swabs (has been initiated) and faecal microbiome.

In recent years, the number of transfusions has declined dramatically in several countries. The blood centres are thus challenged to discover new business opportunities. Experiences from DBDS show that not only are blood donors willing to participate in biomedical research projects, but many actually consider such participation as an additional motivation for donating blood.

The possibility to participate in research projects is now part of the strategy for the Danish Blood Donor Association in the recruitment of new blood donors. We propose that blood donors who have faced deferral could instead be accepted as research donors; that is, as donors of data and blood samples.

For the study of rare events or phenotypes, many participants are needed. We envision a closer collaboration in the Nordic countries and suggest merging our separate, national efforts in a single Nordic Blood Donor Study.

1B-02-02

PERSPECTIVES AND OPPORTUNITIES FOR THE DANISH TRANSFUSION DATABASE

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The Danish Transfusion Database (DTDB) was founded in 2000, and is one of the official Danish National Clinical Quality Databases. The aim of DTDB is to describe transfusion practice in Denmark, in order to ensure that the treatment complies with current evidence and applicable guidelines.

The Danish Society for Clinical Immunology (DSKI) started to plan the creation of a Danish Transfusion Database in 1995, as the transfusion services in Denmark finally were computerized. The first report came in 2002, and consisted of only four pages. In 2005, new "custom-made" database software was made by Databyrån AB (Stockholm, Sweden). In 2006, reporting of data to Clinical Quality Databases went from optional to obligatory in Denmark. This resulted in 100% national coverage in 2007 for blood transfusion registers and the national diagnosis and procedure register (LPR), and for clinical biochemistry registers some years later.

The main "product" from DTDB has until now been the annual report, which is built up on four indicators.

Indicator 1 is the percentage of hospital admissions and relevant outpatient contacts were a RBC transfusion were given. In 2015, this was the case for 4.3% of the admissions (4.7% in 2014 and 5.2% in 2013). Data can be used to monitor developments at individual hospitals over time, but it is not meaningful to make comparisons between individual hospitals or regions.

Indicator 2: The proportion of hospital admissions and relevant outpatient contacts which are given odd number of transfusions. The indicator was introduced due to the long-standing and inappropriate tradition of giving transfusions "as pairs". The standard is a proportional distribution between even and odd numbers, thus the proportion of admissions transfused with odd numbers of units should be above 45%. We are almost there, as admissions with odd numbers of transfusions increased from 36.4% in 2012 to 44.8% in 2015.

Indicator 3: Percentage of hospital admissions and relevant outpatient contacts where the hemoglobin concentration were measured after RBC transfusion. This indicator has some technical issues, as it is not always possible to determine whether a hemoglobin measurement within the same date was made before or after the transfusion. Therefore, there is only registered a hemoglobin concentration after RBC transfusion for 50% of the admissions. It is expected that electronic transfusion registration, which is planned or even started in most of Denmark, will solve this problem.

Indicator 4: Percentage of hospital admissions and relevant outpatient contacts where the hemoglobin concentration after transfusion were >5.5 mmol/l (9 g/dl). As much as 74.1% of the included patients had a hemoglobin concentration above the recommended maximum of 5.5 mmol/l. This is well above the intended standard of less than 10% of patients, and indicates that three quarters of transfused patients in Denmark are transfused at a hemoglobin concentration higher than the current recommendations. This is probably the explanation why blood consumption in Denmark remains high by international standards.

The future perspectives and opportunities for the Danish Transfusion Database will be discussed during the presentation. This includes plans for automation of data registration, giving the possibility of current data analyses which could be accessed by the clinicians on an ongoing basis in addition to annual reports.

1B-02-03

SCANDAT DATABASE – FUTURE DIRECTIONS?

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Background: The first version of the Scandinavian Donations and Transfusions (SCANDAT) database was created between 2003 and 2004. It was designed to contain all data on blood donors, blood donations, blood component processing and blood transfusions that was electronically available at Swedish and Danish blood banks and transfusion medicine clinics. Since then, the database has been updated once with data complete through 2012, and currently contains information on more than 1.6 million blood donors linked through blood products to 2.1 million transfused patients. For both donors and recipients there is extensive follow-up through a range of health outcomes registers. The data has proven its usefulness in a series of publications. However, over time, the stationary nature of the database and the lack of clinical details have proven to be a limitation. Further development is therefore needed.

Aims: To update the SCANDAT database and improve its utility by including additional clinical detail.

Methods and expected results: The next version of the SCANDAT database is currently being planned. We envision expanding the scope of the database by including more details about patients who have not been transfused, but who underwent type and screen in preparation for surgery or other medical treatment. We will also incorporate detailed data (including blood counts, measures of iron stores, etc.) from local and regional laboratory databases. We will incorporate more details on donor testing, blood processing, antibody testing, and erythrocyte surface antigens, to improve the ability to study administrative aspects of transfusion medicine. To further facilitate studies of

blood donor health and health effects of blood donations, and to allow analyses of biological specimens a closer link will be established with the Danish Blood Donor Study. Lastly, a pilot project has been initiated in Denmark to develop routine procedures for data extraction and processing for regular and cost effective updating of the database. A similar process will be established also in Sweden.
Conclusions: The proposed changes to the SCANDAT database will expand its scope and usefulness. We envision a database that is useful not only for etiologic research, but may also be used for evaluation of blood processing routines, adherence to guidelines, and comparative effectiveness research as well as specific transfusion-related risk assessments. Further clinical detail (e.g. laboratory results) should also improve adjustment and outcome definitions in etiological research studies. Ultimately, a near-continuously updated database will be useful not only by improving its timeliness and generalizability, but will also make it possible to conduct register-based randomized trials, where randomization happens prospectively, but follow-up and outcome ascertainment is done only through available data from the database. This design is being used increasingly and combines the external validity and cost-effectiveness of retrospective studies with the internal validity of randomized trials.

Plasma derived medicinal products and self-sufficiency

1C-03-01
PLASMA SUPPLY MANAGEMENT IN THE COUNCIL OF EUROPE

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Background: Different stakeholders contribute to the management of plasma and plasma-derived medicinal products (PDMP) in Europe - blood establishments, manufacturers of PDMP, patient organisations and donor associations. Important achievements in donor safety and component quality have been reached by the Council of Europe (CoE) programs for blood transfusion, currently led by the European Committee (Partial Agreement) on Blood Transfusion (CD-P-TS) coordinated by the European Directorate for the Quality in Medicines and HealthCare (EDQM). In 2014 the CD-P-TS decided to create a working group (WG) to address current issues of safe and sustainable plasma supply management.
Material and Methods: The WG identified tasks such as assessing supply vs demand, use and need of plasma for fractionation (PfF) in member states (MS), developing tools to assess and support a higher degree of collection, preparation and use of plasma of the required quality to its full potential and promoting sustainable and safe plasmapheresis. The WG has used both available and additionally collected data to obtain a clear landscape. The WG also uses other CoE programmes and activities such as the Wildbad Kreuth symposia on optimal use of blood products and the Good Practice Guidelines (GPG) recently referenced in EU legislation.
Results: The amount of PfF delivered by MS 2001–2013: 0.0–54 l per 1000 inhabitants. In 2013 the average delivery was 9.1 l/1,000 inhabitants, mainly recovered plasma. The volume collected by apheresis was on average 4.2 l (range 0.0–50 l, median 0.4 l) of plasma per 1000 inhabitants with four MS collecting more than 10 l per 1000 inhabitants. Different rules for maximum apheresis collection volume and frequency are practiced in Europe, which differ from those specified in the CoE Guide to the preparation, use and quality assurance of blood components (CoE Guide). PfF is delivered to the commercial fractionation industry, however in a few MS this is conditioned to national delivery of PDMP only, which gives national control of all aspects of the process. Reporting on the use of PDMP to the annual CoE survey was always limited as such data may not readily be provided to CD-T-PS representatives and so the use and need of PDMP vs supply of plasma is difficult to assess. The Wildbad-Kreuth symposium 2013 on the optimal use of clotting factors and normal immunoglobulins resulted in two resolutions adopted by the CoE committee of ministers (COM) in 2015. Although the parameters of use, demand and need for PDMP in Europe are not fully clarified, there is a need for more PfF from Europe. The use of erythrocytes is decreasing and also the number of whole blood collections, limiting the access to recovered plasma. This has increased the interest in plasmapheresis collection. The scientific basis for safe and sustainable apheresis has been reviewed. This has unveiled the need for updating relevant text in the CoE Guide. Knowledge gaps have been identified and described. Research to fill such gaps will be promoted.
Conclusions: In cooperation with different stakeholders, the WG will support improvement of plasma supply management in Europe. Future recommendations based on scientific evidence will be provided as a basis for revising the CoE Guide.

1C-03-02
TOWARDS PLASMA SELF-SUFFICIENCY – THE PROCESS AND THE STATUS IN DENMARK

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Plasma-derived medicinal products (PDMP) such as human immunoglobulin (IgG) and albumin are included in WHOs list of Essential Medicines. WHO and the Council of Europe recommend a national self-sufficiency strategy for PDMP. Due to a sharp decline in the transfusion need of red blood cells and thus a corresponding decline in the intake of recovered plasma together with a growing need for PDMP throughout the Western world, most Western countries do not produce enough national plasma for outweighing their PDMP needs. Taken together, the European Union is far from being self-sufficient, and the Danish situation is typical. In 2007, Denmark was largely self-sufficient in IgG and albumin, but today less than half of the required Danish plasma is provided to meet an increasing national need for IgG. The situation is due to the declining amount of recovered plasma and an insufficient production of source plasma on top of a rapidly growing need for IgG for medical treatment of mainly neurological diseases. Since 2004, Danish plasma has been fractionated into PDMP for Danish patients in a closed circuit via a contract with an international pharmaceutical company. Due to the developing gap between the Danish need of PDMP and the national plasma production, Denmark is now considering various strategies. These strategies take into account the Danish plasma from whole blood and plasmaphereses. A brief review of the Danish situation and the Danish legal framework in the area will be given. Essentials of the strategies under considerations, such as self-sufficiency and security of supply of PDMP, a regulation of the IgG usage as well as important issues related to the Danish donor base, blood bank productivity and overall economic issues will be discussed.

Blood supply and management in the future

1D-04-01
WHERE AND HOW TO COLLECT BLOOD IN A CHANGING WORLD

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Background: In the past Denmark has always had sufficient numbers of non-remunerated donors even though the country's use of blood traditionally has ranked amongst the highest in the World. Due to changes in demography it has been foreseen that the use of blood - *ceteris paribus* – will increase by more than 50%. This projection together with the shutting down of many small hospitals that were hosting fixed blood collection sites resulted in a change from an all fixed site-blood service to increased use of mobile units accounting for 34% of WB collections in 2015. In spite of this and although the use of blood has been halved during the last decade it has been harder to get the sufficient number of donors, especially during daytime.
Aim: To understand the causes of the change in donor behavior and thereby being able to respond adequately.
Methods: Literature, internet-search and Statistics Denmark were used to map changes in the society during the last couple of decades.
Results: The following changes in society may cause the described problem: (i) Demography: A relative decrease in population between 17 and 67 that are eligible to donate; (ii) Urbanization: A decrease in population – especially those eligible for

blood donation – in peripheral, rural and intermediate municipalities (> −10% since 2000)–making mobile units less effective; (iii) Increased commuting (10–15% since 2000): Due to closure of local businesses and an improved infrastructure a greater proportion of the working force commutes and for longer distances, especially from peripheral and rural municipalities – making mobile collections less effective during daytime; 4. Increased specialization of the work force: Outsourcing of production to other countries and increased use of digital and robot technologies imply that every worker has a very specialized function which together with tougher competition means that the employee is not allowed time off work to donate blood as was the custom in the time of the assembly belt – making both fixed site and mobile collection less effective during daytime.

Conclusion: Society has changed and is still changing for parameters such as demography, urbanization, commuting patterns and possibility to get time off from work, all of which influence the planning and efficiency of blood collections. Collections therefore must take place before or after working hours, either near the work place or near the donor's home. It may be difficult for the blood industry to recruit and keep staff with the right qualifications for such working hours, especially in a nearly full-employment society where the working force is decreasing due to changed demography. The most cost-effective response to the described developments is bigger fixed collection centers in urban areas. However, this may clash with political wishes in a public governed health care system.

1D-04-02

CENTRALIZED BLOOD ESTABLISHMENT – WHAT ARE THE ADVANTAGES

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In Europe, blood establishments have three basic organizational models. In the most centralized model there is only one operator in the whole country. This model has been adopted in Finland (Finnish Red Cross Blood Service, FRCBS); France (L'Établissement français du sang, EFS); The Netherlands (Sanquin) and The United Kingdom (English NHSBT; Scottish National Blood Transfusion Service; Welsh Blood Service). In Germany and Switzerland there are independent operators under the same umbrella organization (Red Cross). In Denmark, Sweden and Norway there is a localized hospital driven model with several dozen local blood establishments in Sweden and Norway and a lesser number in Denmark. There are only anecdotal studies where performance, quality and availability of the blood products, costs and customer satisfactory are compared in the centralized and decentralized blood establishment models. However, some conclusions can be made on the basis of general knowledge and experience.

All blood establishments face varying degrees of direct and indirect costs involving data management, equipment, materials, staffing, support functions and facilities. The economy of scale is a clear advantage in the centralized model. When the volume arises, the per-unit fixed costs decrease. The centralized model gives good opportunities to optimize procurement processes and to gain low prices for consumables. There are also better possibilities to adjust the human resources to the changes of the needs. In addition to the better economy the centralized organizations are likely to be better positioned to invest in research activities that enhance blood products quality and transfusion medicine. Centralized donor management gives tools for long term national planning and rapid response to the changes in the need for blood products. It also compensates the geographic differences in the availability of donors. Nationwide supply chain management including national inventory management secures the availability of blood products to all hospitals and helps to avoid local supply shortages. It reduces the risk associated with external shocks on the supply of or demand for blood products. Centralized blood establishments have a good possibility to take a strong role in the enhancement of good transfusion practices by education, quality issues, big data analysis (benchmarking, trend analysis) and policy making.

The decentralized model has also its benefits. Local blood establishments may provide added value by maintaining close relationships with the hospitals they serve. They may have more full-time staff locally which gives them the opportunity to take part in joint activities with the customer they serve beyond the simple delivery of blood. Medical and scientific consultations may also more easily flow in both directions in the decentralized model.

Academy Day

Donors and donation

2A-01-01

TEENAGE BLOOD DONATION: TRENDS, ADVERSE REACTIONS AND IRON BALANCE

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Background: Young (16- to 17-year-old) volunteers contribute substantially and disproportionately to the blood supply in the United States, accounting for less than 3% of the general population but about 10% of allogeneic whole blood collections. This translates into over 1.3 million donations each year at high schools, which is even more than college-age donors. Donating blood, while quite safe overall, has some attendant risk and teenage donors are the most susceptible to adverse reactions and injuries. Moreover, many adolescents, especially girls, have low iron stores even though they have adequate hemoglobin levels, predisposing them to the potential adverse consequences of iron deficiency after blood donation.

Aim: To describe demographic trends in blood donation and the risk of adverse reactions and iron depletion among teenage (16- to 17-year-old) blood donors compared to older donors.

Methods: Review of demographic data and studies on blood donation by 16- to 17-year-olds.

Results: Many states lowered the minimum donation age to 16 years largely in response to the increasing demand for blood transfusion; subsequently, collections from 16-year-olds increased rapidly after 2007. Since then, however, blood utilization has decreased each year and blood collection has fallen to its nadir in 2013, 21% lower than 2008. Yet collections from 16-year-olds continued during this time, resulting in an influx of 16-year-olds but a concomitant decrease in older donors. Most states require parental permission for 16-year-old blood donors, but not for 17-year-olds, even though both groups are at higher risk of adverse reactions compared to adults. The odds of syncope-related injury after donation for a 16- or 17-year-old are 2.5-fold higher than 18- and 19-year-olds and 14.5-fold higher than adults 20 or older, even after taking into consideration first-time donation status and other predisposing factors. Moreover, iron deficiency is prevalent among teenagers and further depletion with blood donation should be avoided by measures used either alone or in combination, such as reducing the frequency of donation, increasing the interdonation interval, ferritin testing and iron supplementation. To prevent reactions, eligibility criteria to select donors with a higher estimated blood volume (EBV>3.5L) avoids the loss of more than 15% total blood volume with a standard 525 ml whole blood donation. This approach decreased symptomatic reactions and syncope among young donors compared to an unselected group, and the effect was most evident among the youngest female donors. However, the intervention had no effect on syncope-related injuries. Measures such as predonation water ingestion and muscle tension exercises during collection have demonstrated some benefit in controlled trials but have had less consistent results in routine practice.

Conclusion: Teenage donors continue to contribute substantially and disproportionately to the blood supply despite their higher risk of adverse reactions and possible consequences of iron depletion with blood donation. Interventions to reduce the risk of reactions and mitigate iron deficiency are supported by research studies and large operational trials in blood centers, but have not been universally implemented. Further measures to prevent donation-related reactions and iron depletion could protect adolescent blood donors.

2A-01-02

THE OLDER DONOR

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Background: The upper age limit for blood donation differs in various countries, due to concerns about donor safety. With the baby boomer generation now aged between 53 and 71 years, there is potential for increased demand for blood products in many countries. However, this bulge in the aging population also brings an increase in the number of healthy older people in the general population who could contribute to the blood supply. In recent years many countries have reviewed their policies regarding the upper age limit for donation. Evaluation of the impact of both changing population and donor demographics and revision of donor age criteria on the blood supply may assist in better planning to meet future patient needs for blood.
Aims: We provide an overview of policies, evidence of the safety of donation in older individuals, and an analysis of the contribution that older donors can make to the blood supply.
Methods: We reviewed policies in 12 countries that are part of the Biomedical Excellence for Safer Transfusion Collaborative (BEST), and published studies on the safety of donation in older donors. We evaluated the contribution that older donors make to the blood supply at Canadian Blood Services (CBS), which provides blood for all of Canada except the province of Quebec.
Results: The upper age limit for regular whole blood donors ranges from 69 to 70 (Japan, Australia, Belgium, France, the Netherlands, Singapore) to 75 (New Zealand); in the last few years, blood centers in several countries have removed the upper age limit (Canada, England, Ireland, Germany) while many blood centers in the US have long had no upper age limit for donation. Data from the US, Canada, and Germany demonstrate that healthy older individuals can safely donate, with reaction rates comparable to donors in the 30 to 60 year old cohort. The majority of the data evaluates regular donors, rather than first time donors. In Canada, using population prevalence data for deferrable diseases/conditions, we estimated that removing the upper age limit of 71 would theoretically increase the population base eligible to donate by close to 9%. In practice, over a decade after removing the upper age limit, 1.5% of all whole blood donations at Canadian Blood Services are made by donors past their 71st birthday. These donors were predominantly male (65% vs 51% for all CBS donors) and made more annual donations than younger donors (3.0 vs 2.3 for males, and 2.4 vs 1.8 for females). Most of the older donors were between 71 and 75.
Summary/Conclusions: There are more healthy older individuals than ever before. Many donors would like to continue donating into their eighth decade, and perhaps beyond. There is increasing evidence that donation is safe in older individuals, and that they can make a significant contribution to the blood supply.

2A-01-03

THE DEFERRED DONOR

V Compernolle

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Donor selection criteria remain an important step in ensuring the safety of blood products and the wellbeing of donors. Donor deferral has the advantage of rapid implementation in case of emerging infectious diseases, and – at first sight – limited costs. The level of effectiveness of each individual measure is however not always clear. Deferral may also come with hidden costs. Several studies demonstrated the effect of donor deferral on the likelihood of the donor to return for a next donation. In an world in which the word-of-mouth becomes more important due to the availability of social media, in which donors more frequently and more critically question the justification of their deferral, the need to strengthen our reasons of deferral with evidence and to more clearly communicate them to the donor population and the general public grows. Moreover, educating donors and making tools available to promote pre-session self-deferral will decrease onsite deferral and results in advantages for both donor and blood bank.

Transfusion therapy: Focus on haemolytic disease of the foetus and newborn

2A-02-01

HAEMOLYTIC DISEASE OF THE FOETUS AND THE NEWBORN (HDFN): THE LABORATORY PERSPECTIVE

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Haemolytic disease of the foetus and the newborn (HDFN) is caused by maternal red cell alloantibodies of the IgG class that are actively transported across the placenta and destroy foetal erythroid cells carrying the antigens involved. Severe HDFN due to anti-D antibodies is still a matter of clinical concern despite the introduction of anti-D immunoglobulin (Ig) prophylaxis. In different European countries, the incidence of maternal anti-D alloimmunization remains at 1 to 1.5 per cent in D negative women mainly because of failures of adequate anti-D Ig prophylactic administration or occult antepartum transplacental haemorrhage. About 10 percent of the immunized women have babies that are severely affected *in uterus*, and the management of such pregnant women requires intensive monitoring to identify the foetuses that are at risk of HDFN as well as the optimal time for intervention in order to prevent foetal anaemia and the risk of hydrops foetalis. Anti-c, anti-E, other anti-Rh antibodies and anti-K can also cause severe HDFN. However anti-K carries the highest risk to induce disease in an antigen-positive child, followed by anti-c.
Accurate information concerning the presence and nature of red cell alloantibodies in the plasma of pregnant women is essential for the effective management of potential HDFN. Screening in the laboratory is designed to provide this information to clinicians. Serological methods are effective in antenatal screening programmes because they are capable of detecting the vast majority of red cell alloantibodies that can cause HDFN. Screening is usually undertaken in blood transfusion laboratories because the techniques used for screening for HDFN are the same as those used in pre-transfusion testing. However, there are also some important differences since the clinical significance of red cell antibodies may vary in these two situations. In practice, screening tests for HDFN and pre-transfusion tests are complementary because the presence of a red cell antibody in a pregnant woman may be important not only for HDFN but also for the selection of blood should transfusion be indicated for the mother or the foetus. Laboratory protocols are therefore designed to take these overlapping requirements into account.
In the past decade, non-invasive monitoring of high-risk cases by laboratory testing and by ultrasound-based techniques to evaluate the presence of foetal anaemia have replaced invasive procedures. Foetal D genotyping from maternal plasma is now systematically performed in some European countries to guide the administration of IgG anti-D. Foetal C, c, E and K genotyping is also currently feasible.
Maternal alloantibody titres are still used to predict the risk to the foetus and, in addition, some countries also use in vitro antibody-dependent cellular cytotoxicity biological assays. Recent studies have described a significant association between antibody glycosylation patterns and foetal outcomes. Low anti-D fucosylation correlated significantly with low foetal-neonatal haemoglobin levels, thus with increased haemolysis, suggesting that IgG-fucosylation can be very useful to predict clinical severity with more accuracy.
Although much progress has been made we still have a lot to know and to investigate about alloimmunization risk factors, preventative therapies, and treatment strategies in HDFN.

2A-02-02

HDFN: THE MATERNAL-FETAL MEDICINE/OBSTETRIC PERSPECTIVE

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No Abstract available

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NEONATAL MANAGEMENT AND OUTCOME IN HEMOLYIC DISEASE OF THE FETUS AND NEWBORN

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Implementation of Rhesus (D) immunoprophylaxis in 1965 has led to a drastic decrease in incidence of hemolytic disease of the fetus and newborn (HDFN). Nevertheless, due to failure of pregnant women to obtain the prophylaxis and in a minor extent due to failure of the prophylaxis itself, anti-D is still the most commonly implicated antibody in HDFN. Other clinically relevant antibodies associated with severe HDFN are anti-Kell and anti-c. Antenatal management of HDFN is based on the treatment of fetal anemia and prevention of fetal hydrops with intrauterine red cell transfusions (IUT). Since the introduction of IUT in 1963 by Sir William Liley, perinatal survival in HDFN has nowadays increased above 90%. Postnatal management in neonates with HDFN is based on the treatment of hyperbilirubinemia and prevention of kernicterus and includes mainly the use of phototherapy and exchange transfusions. In the last decade the use of intravenous immunoglobulin (IVIG) is increasingly being advocated, but several randomized trials with low-risk of bias, failed to show a beneficial effect of IVIG in reducing the rate of exchange transfusions. Postnatal treatment in neonates with HDFN also consists of treating or preventing early and late hypo-regenerative anemia using top-up transfusions and supplements such as folic acid and iron. The additional use of erythropoietin treatment has also been studied in several small studies. A randomized controlled trial is urgently needed to determine the beneficial effect of erythropoietin in reducing the need for late top-up transfusions. In addition to anemia, other hematological complications such as thrombocytopenia, coagulation disturbances, leucopenia and iron overload have been reported. In this presentation, I will provide an overview of the hematological morbidity in HDFN due to red cell alloimmunization and summarize the neonatal treatment options.

Organisation and quality/ clinical governance

2B-03-01

THE ROLE OF THE NATIONAL BLOOD TRANSFUSION COMMITTEE

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Many countries have established national blood transfusion services in line with World Health Organization (WHO) guidelines and recommendations. However, according to WHO, few countries have developed policies and guidelines on the clinical use of blood and products which are being effectively implemented. This review outlines the WHO recommendations for a National Committee on the Clinical Use of Blood and describes the establishment of a National Blood Transfusion Committee (NBTC) in England and the first 16 years of its work. Although it is difficult to document the value of the NBTC, the improvements in transfusion safety in hospitals and the marked reduction in blood use across a large country would almost certainly not have happened to the same extent without it. The inclusive membership of the NBTC of Royal Colleges, professional organisations, NHS Blood & Transplant as the national blood supplier and NHS senior management have been key to providing impetus to the implementation of the output of the NBTC. The linkage to hospital transfusion practice via the RTCs and HTCs has proved very beneficial for the dissemination of its work. Despite the excellent progress, there is much for the NBTC to do in the future to ensure that transfusion practice is as safe and as effective as possible.

2B-03-02

HOSPITAL TRANSFUSION COMMITTEES – COMMON GOALS, PROBLEMS AND PITFALLS

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Across the globe there are many different standards that provide guidance on the goals and activities of hospital based transfusion medicine committees but these are not always interpreted or implemented in the same manner. This session will highlight some of these standards and undertake a high level review of the similarities and differences between various jurisdictions regarding the intent, structure and activities of hospital based transfusion medicine committees. The attendees of this session will be asked to participate in an interactive survey process to compare their own hospital transfusion committee's structure and function with a variety of the highlighted recommendations. In addition, common deficiencies and pitfalls identified by various hospital transfusion medicine committees or accrediting bodies will be discussed and examples of solutions that have implemented with and without success will be provided to attendees. There will also be some discussion of how new technologies and changes to transfusion medicine practice may require the evolution of transfusion medicine committees in the years to come.

2B-03-03

CLINICAL GOVERNANCE IN THE BLOOD SERVICE

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Background: Just as in clinical care the principles of Clinical Governance also apply to Blood Services. These will complement regulatory requirements such as Good Manufacturing Practice to ensure that patients and donors receive the highest level of care with the best outcomes. As such Clinical Governance is essentially a quality framework for the delivery of clinical care.

The concept of clinical governance emerged from the National Health Service (NHS) in the UK, as a system through which NHS organisations are accountable for continuously raising the standard of clinical care. This requires an organisational culture where clinicians feel able to raise issues and make improvements which should be both corporate and consistent throughout the organisation.

Aims: There are several key features of good clinical governance. As described by Harrison there are 10 pillars of Clinical Governance. Leadership and accountability of both clinical and non-clinical staff are important, with the ultimate responsibility for clinical care resting with the chief executive but being promoted by all senior staff and clinicians. The reporting, collection and analysis of data in a Quality Management System, along with an honest and open culture and a willingness to learn from errors are also critical. Prompt reporting and robust investigation of incidents and a clinical audit programme, both with outcomes resulting in changes to practice are required. Evidence-based decision making is essential, as is learning from near miss events and an understanding of Human Factors. Clinicians shouldn't wait for an event to happen but should learn from near miss events and be proactive in identifying and managing potential risks within an organisation.

For a Blood Service to know if Clinical Governance within an organisation is 'working' a system of assurance is required. Usually this involves a corporate wide system to report to the Board or senior staff on the different elements of Clinical Governance. For example reports on adverse events and measures of safety should be developed and regularly reported. Clinical audits should be regularly undertaken. Risks should be assessed and recorded with mitigations implemented. Systems for monitoring complaints or claims against an organisation are also needed. Horizon scanning for risks, external guidance and to inform evidence based medicine are good practice. Appraisal or similar methods for assuring that individuals employed in an organisation are up to date and fit to practice are ideal, as are processes for dealing with individuals where this is not the case.

The systems and processes that exist within NHSBT to deliver these outcomes will be described.

Summary/conclusions: The overall is objective of having a Clinical Governance framework within a Blood Service is to improve services and prevent adverse outcomes in both donors and patients. Delivery of a good system requires a number of elements to be put in place and fundamentally a culture where all staff are willing to learn and improve outcomes for donors and patients.

Immunohaematology

2B-04-01

NEW LABORATORY TOOLS FOR IMMUNOHAEMATOLOGY: CORRELATING FLOW CYTOMETRY AND MOLECULAR RESULTS

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Background: Flow cytometry (FC) has traditionally been used to analyse subpopulations of leucocytes in quality control of stem cell grafts and in the diagnosis of lymphoma and leukaemia although there are numerous other applications in use for this versatile method today. In transfusion medicine, it has mainly been utilized for detection and semi-quantification of various blood group antigens as a complement to traditional serology and for quality control of blood components. There are some tests where the standard methods used in transfusion medicine are not sufficient and FC is a good alternative method. Examples of this are quantification of anti-D in immunized women and phenotyping of Human Platelet Antigens (HPA). Also the detection of fetomaternal haemorrhage by FC is a relatively simple and a more precise method in comparison to the more subjective Kleihauer-Betke test.

FC has mainly been used in a reference laboratory setting since the equipment is relatively expensive and the technique requires experience and knowhow when setting up experiments/templates. Today, when flow cytometers are becoming smaller, cheaper and more user friendly and are available in many routine laboratories, the method should be considered as a valuable tool to use in addition to serology and genomic typing.

Aim: To give an overview of how FC can be useful in a transfusion medicine setting as a complement to both traditional serology and molecular testing.

Discussion points: FC has the advantages of a higher sensitivity for some antigens than serology and the ability to easily distinguish between two or more populations of cells, which makes it a useful tool in many clinical settings. Mixed field result in tube testing or gel columns may be due to a weak subgroup, transfusion or the patient being a natural chimera. In ABO minor incompatible stem cell transplantations, uptake of glycolipids with A and/or B specificity from plasma to donor derived group O erythrocytes may cause problems in ABO typing and give rise to the question whether this is a relapse or not. By FC it can easily be established if it is a true mixed field reaction or a weak expression.

The combination of molecular testing and the detection of surface antigens in e.g. resolving ABO discrepancies has been very helpful in our laboratory [Hult &tOlsson, Transfusion. 2010;50(2)] We have also used FC to screen for HPA-1a platelet donors to ensure a supply of these products. Furthermore, FC can be used to find crossmatch negative platelets for platelet refractory patients demanding HLA- or HPA-typed units.

FC is a useful tool in clinical routine analysis as well as in research focusing on transfusion medicine. In our laboratory routine work up of patient samples sometimes transitions into the field of research but with focus to solve clinical problems. Many laboratory methods are used in combination and FC has proven to be a valuable part of this.

2B-04-02

MANAGING A RARE DONOR PROGRAM-THE IMMUNOHAEMATOLOGY LABORATORY PERSPECTIVE

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Genetic disparities between blood donor and recipient can cause antibody formation by the recipient against donor's erythrocyte antigens. Alloimmunization is a significant complication for patients who require multiple RBC transfusions, as it complicates serologic investigations and makes the selection of compatible blood difficult, expensive and time consuming. Alloimmunization rates can be very high in selected groups of regularly transfused patients such as those suffering from thalassaemia or sickle cell disease (SCD). The supply of compatible blood may become particularly challenging for patients developing alloantibodies to high-prevalence antigens or multiple antibodies to common antigens. The Immunohaematology Reference Laboratory (IRL) is a high specialized area in charge of: identifying challenging antibodies, using multiple methods of analysis and a broad inventory of reagents and cells not easily available; finding compatible blood units for patients with complex alloimmunization and designing appropriate transfusion strategies for these patients also in cooperation with international programs. As the prevalence of blood group antigens varies among different ethnic groups, the definition of "rare blood donor" varies from country to country. According to the American Rare Donor Program standards, an individual is recognized as a rare blood donor when is negative for high-prevalence antigens with a frequency less than 1:1000 or negative for multiple common antigens. Prompt availability of compatible units for patients with complex alloimmunization requires access to an inventory of extensively typed blood and to a database of rare donors. The key factors for rare donor provision are: routinely carry out programmes of typing red cell antigens in large cohorts of donors with automated high-throughput molecular or serological methods; procedures to identify rare donors when a routine antibody screening test is positive for antibodies to high-prevalence antigens; donor selection using family members, especially siblings, of patients immunized against high-prevalence antigens; enrolment of patient negative for high-prevalence antigen into autologous or allogenic RBC storage programs and rare donor panels. The IRL is, therefore, involved in the whole process from resolution of complex cases of red cell immunization to the identification and provision of compatible blood. In order to achieve this role, the IRL have to adopt high quality standards and have specialized laboratory staff. In addition, it is necessary a close collaboration between the IRL and clinicians for management of patients who need rare blood units.

2B-04-03

CHALLENGING RED CELL SEROLOGY CASES

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Due to pregnancy or blood transfusions patients can develop antibodies against blood group antigens (alloimmunization). To prevent hemolytic or delayed transfusion reactions an antibody screen is performed before blood transfusion. In most cases this test will show a negative result, however in some cases a positive result is obtained. Antibody identification will mostly result in a distinct antibody specificity, but in certain cases the result cannot be obtained by routine immunohematological laboratory work-ups. Such cases are mostly referred to local or national reference laboratories where additional tests are undertaken. These tests include: testing at different temperatures and in different milieus, the use of rare blood cells and serums, the use of test cells treated with enzymes and chemicals, soluble recombinant proteins and blood group genotyping. The cause of the observed reactivity within such samples can have many different backgrounds, for instance a combination of antibodies (e.g. anti-s, anti-e and anti-Jk(a)), antibodies against high frequency antigens, unbound erythrocyte auto-antibodies and artifacts such as antibodies against substances in the stabilization solution or drugs such as anti-CD38 (Daratumumab or Darzalex). The process of identifying the underlying cause of reactivity is sometimes very challenging and thus the knowledge of serological characteristics of antibodies and antigens is fundamental. Antibody identification will however never be the sole important issue of the serological problem solving. It is equally important to know how to transfuse such patients. For this purpose, the information on the clinical relevance of the antibodies is very important.

TTID

2C-05-01

NEW INFECTIOUS EPIDEMICS: THE GLOBAL HEALTH VIEW

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The 2014-2016 Ebola outbreak in West Africa indicated that despite previous development of promising Ebola vaccine candidates the products were not available in time, due to manufacturing issues and regulatory preconditions not yet fulfilled. Despite international regulatory cooperation with high urgency Ebola vaccines became available only at the late declining stage of the epidemic. Similar problems were encountered with Ebola diagnostics and therapeutics. To be better prepared for future epidemics or health emergency situations where there are no, or insufficient, preventive and curative solutions, WHO was requested to initiate a research and development blueprint project. It aims to reduce the time between the declaration of an international public health emergency and the availability of effective tests,

vaccines and medicines that can be used to save lives and avert crisis. After priorisation of potential key pathogens the already existing specific products (vaccines, diagnostics, therapeutics) are analysed (landscape analysis), followed by a specific roadmap for research and development. This roadmap also aims to anticipate evidence needs to inform regulatory view and policy development. End of 2015 the first list of key pathogens was developed, followed by annual updates. The 2017 list of key pathogens includes Arenaviral haemorrhagic fevers (e.g. Lassa fever), Crimean Congo haemorrhagic fever (CCHF), Filoviral diseases (e.g. Ebola, Marburg), Middle East Respiratory Syndrome Coronavirus (MERS-CoV), other highly pathogenic coronaviral diseases (e.g. SARS), Nipah Virus, Rift Valley Fever (RFV), Severe Fever with Thrombocytopenia Syndrome (SFTS) and Zika. MERS-CoV has been chosen as first candidate for development of the specific roadmap; based on the experience gained with this pilot project in international cooperation the roadmaps for the other candidates will be adapted and followed in order to be better prepared for potential future emergence of these pathogens.

2C-05-02

MODELLING RISKS OF EMERGING INFECTIOUS DISEASES RELEVANT TO BLOOD TRANSFUSION

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In recent years an increasing number of infectious diseases emerged. These infectious diseases may pose a threat to the blood supply, either by local epidemics where the donor population is directly exposed to the infectious disease, or by travelling donors who visit an outbreak region and potentially transmit infection upon returning home. The ability to quantify such risks can support decisions concerning the necessity of additional safety measures, or refraining thereof.

The common approach in any risk assessment is to start from the undesired event and systematically reason backwards throughout the risk pathway. By the risk pathway we refer to a sequence of events leading to the undesired event under consideration. In our case the undesired event is a transfusion recipient becoming infected. As the blood transfusion chain consists of directly linked steps, the assessment of the risk pathway is rather straightforward: a transfusion recipient gets infected by an infectious blood product containing a sufficient level of infectious agent; this blood product comes from a donation from an infected donor with a sufficiently high concentration of infectious agent; this requires a donor to donate at a defined peak of the infection; and this implies that the donor has to be fit enough to donate whilst being infectious. Where for blood components such an assessment is straightforward, for plasma products for example, such an assessment would require additional information on the production process, as this determines the composition of the plasma production pool and the likelihood and extent of individual end products being infected.

Breaking up the process throughout the risk pathway allows defining individual steps that can be modelled in a meaningful and communicable way, and incorporated in the overall risk assessment. The best known risk model in blood safety is the 'window period model' which allows calculating the probability that an infected donation is missed by a screening test. The idea is that there is a fixed rate at which donors become infected and that up until the moment that the recently infected donor has a sufficiently high concentration of infectious agent in his or her blood, detection of the infection by screening fails. The length of the window period depends on the viral doubling time and the sensitivity of the screening test. The probability of missing a recently infected donor despite screening is equal to the product of infection rate and window period length.

In this talk the individual steps in the risk pathway of transfusion transmitted infectious diseases will be explicitly discussed for the risk of transmission from local outbreaks and the risk of transmission by travelling donors who visited a remote outbreak area. In addition, some examples will be provided on an evaluation of recent outbreaks using the European Up-Front Risk Assessment Tool (*EUFRAT*, available from http://eufrattool.ecdc.europa.eu/), which was developed specifically for the quantification of the risk of transmission of infections by blood transfusion.

2C-05-03

EFFECTIVENESS OF PRE-DONATION HEALTH AND TRAVEL SCREENING IN REDUCING THE RISK OF TTIS

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Early in the history of transfusion it became evident that blood can transmit infectious diseases from the donor to the recipient. The first intervention to mitigate this risk was the screening of blood for syphilis. Later, selection criteria and screening for hepatitis B were introduced to decrease risk of hepatitis. However, it was after the dawn of the HIV epidemic that the focus on donor selection increased dramatically. With the HIV scandals in the 1980's, the competent authorities in several countries were blamed for not reacting in time in their wait for firm evidence. As a reaction, the precautionary principle gained a strong influence on decisions regarding the blood system. The principle seeks to mitigate risk through early intervention prior to definitive evidence. How does the precautionary principle affect donor selection, blood supply and expenses today?

For many infections testing is not possible or feasible and instead we rely on donor selection and thus accurate responses from donors to the donor health questionnaire (DHQ) filled before donation. One problem with selection criteria is that we defer donors based on risk and, consequently, we defer many more than are infected. This is different from the test-based discarding of products where only infected or a small fraction of false-positive donors are deferred. Only necessary selection criteria are to be implemented, but how do we define these? Last year we saw the emergence of a truly complicated behavioural criteria to mitigate the risk of Zika virus transmission in non-endemic areas: defer women, who have had sex with a man within 28 days of donation, if the man has visited areas with Zika virus transmission during 3 or 6 months before the sexual contact. Several countries implemented the criteria whereas other countries performed risk assessments concluding that the criteria can be omitted due to an extremely low risk of Zika virus transmission to pregnant women given the assumptions are correct. Have we implemented too many criteria for our donors and do we risk that relevant questions are overlooked by the donors/staff because of too much information?

The DHQ only fulfils its role if the donor understands the questions, remembers relevant behaviour and discloses this information correctly. The level of compliance with DHQ is not known for most items, though surveys have established that the compliance is far from 100%. We tend to perceive criteria as definitive and efficient but how often do blood donors ignore to disclose relevant information? And are criteria violated due to lack of understanding of low-quality questions or do the donors perform self-assessment of risk because of criteria perceived as irrelevant?

Patient Blood Management

2C-06-01

MAKING TRANSFUSION DECISIONS IN CRITICAL CARE

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Anemia is frequent in critically ill patients and is associated with poor outcome. As a consequence, around 30% of critically ill patients receive red blood cell (RBC) transfusion during their stay in intensive care unit (ICU). Nonetheless, RBC administration is associated with adverse events. Altogether, this makes its crucial to define the optimal transfusion strategy in order to avoid unnecessary RBC transfusion and to provide adequate oxygen delivery to the tissues.

The landmark randomized trial on transfusion in critically ill patients, that led to major changes in transfusion practice triggered transfusion only on hemoglobin threshold. However, in this trial only 12% of assessed patients were randomized, questioning the generalization of its results. Furthermore, in this trial didn't considered parameters that may reflect anemia intolerance were not consider in the transfusion to transfuse. Tolerance to anemia may be difficult to evaluate in sedated critically ill patients and advanced makers of tissue hypoxia may be useful in RBC transfusion decision-making process in this population.

Transfusion triggers and parameters to consider in the decision to transfuse depend on the setting. In case of massive bleeding, transfusion decision is made on hemodynamic parameters, vital signs and when possible on bleeding volume estimation. In anemic hemodynamically stable patients, hemoglobin remains the primary trigger for transfusion. In the less severe or in the youngest critically ill patients, a restrictive transfusion strategy based only on hemoglobin level (transfusion for a

hemoglobin threshold of 7 g/dl) has shown to be associated with a decrease in mortality in comparison with a liberal transfusion strategy (hemoglobin threshold of 10 g/dl). This threshold of 7 g/dL is also safe in patients with gastro intestinal bleeding. In patients with acute ischemic heart disease, where anemia may worsen myocardial ischemia, some large observational studies support the benefit of a higher hemoglobin threshold (between 9 and 10 g/dl). Therefore, patients at risk of myocardial infarction, including elderly and cardiovascular patients, should be monitored for cardiac tolerance of anemia and transfused consequently.
During septic shock, a hemoglobin threshold of 7 g/dl is considered adequate. Nonetheless, none of the available studies addressed the benefit of transfusion in patients with an important decrease in tissue oxygen delivery. In this setting, venous oxygen saturation may be considered as a transfusion trigger as well.
In onco-hematology patients and in patients with traumatic brain injury, the optimal transfusion strategy remains unknown. Additional parameters should be taken into account in the transfusion decision and other transfusion triggers need to be investigated.
In conclusion, hemoglobin level is the main trigger for RBC administration in critically ill patients. Transfusion practices have shifted from liberal towards restrictive strategy, making the monitoring of anemia tolerance an important element in transfusion decision. Identification of comorbidities and conditions, impacting on oxygen transport and delivery to the tissues, should be integrated in clinicians' transfusion decision-making process. Research evaluating the benefit to consider additional triggers to hemoglobin threshold in the decision to transfuse RBC in critically ill patients is warranted.

2C-06-02
EUROPEAN COLLABORATIVE INITIATIVES IN PBM
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Patient blood management (PBM), as defined by WHO, is a patient-focused, evidence-based and systematic approach to optimise the management of patient and transfusion of blood products for quality and effective patient care. It is designed to improve patient outcomes through the safe and rational use of blood and blood products and by minimising unnecessary exposure to blood products. Important elements of PBM are optimising of haemoglobin concentration without blood transfusion, minimising blood loss and managing anaemia, especially by use of evidence based transfusion guidelines. PBM is considered important by WHO and hospitals are urged to prioritise PBM initiatives.
Local PBM initiatives differ substantially. In a Danish region the majority of PBM initiatives over the last 10 years have been based on education and enforcement of national guidelines. Evaluation of the effect of these initiatives indicates that education alone can be an effective and durable method of reducing unnecessary transfusions. Another example of local initiatives is an intensive combination of educational interventions for 6 months at one hospital where a large reduction of red blood cell transfusions was achieved in a mixed medical population.
To improve implementation and knowledge of PBM, benchmarking of experiences between hospitals and countries seems rational, and several initiatives regarding PBM are being undertaken in Europe.
Patient Blood Management in Europe (PaBloE) is a working group of the European Blood Alliance (EBA) that was established in 2014 by bringing together specialists with an interest in PBM from university hospitals in Europe and associated blood services. Its objectives are to derive good practices in PBM based on the experience and expertise of the participating teams, and to develop ways to implement and strengthen these practices in the participating hospitals. Within this group several surveys have been performed relating to blood product consumption and knowledge of PBM principles and practices among clinicians. The surveys have shown variation in transfusion rates between the participating hospitals, and variable implementation of PBM activities and monitoring of transfusion practice. Furthermore, knowledge about PBM among clinicians was found to be rather poor. At the present the group is auditing use of blood in haematology and management of preoperative iron deficiency anaemia.
The European Patient Blood Management Project under EU is another working group in Europe with a strategy to help national authorities to disseminate and implement PBM in hospitals across the EU.

2C-06-03
MAKING CLINICAL AUDIT WORK FOR YOU
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Clinical audit is the systematic, critical analysis of healthcare delivery comparing outcomes and practice against defined, evidence-based guidance. It enables care providers and patients to know where their service is doing well and where there could be improvements. In this presentation I will discuss how quality is defined: there could be an agreed standard devised by a regulatory body, guidelines issued by medical associations or societies, or simply local agreement on the best way to do things. Auditing practice is never successful until quality is defined and that definition is agreed by those whose work is to be examined. Having gained such an agreement, the next stage is to define what information will be needed to measure the level of performance, and examples will be shown together with suggestions for testing out data collection methods. Having collected data, that has next to be turned into information that will be useful to healthcare providers – they must understand where care can be improved, why this is necessary and, above, all how such improvements could be made. The presentation will cover the essentials of effective and timely feedback. But defining quality, collecting data and feeding back information leaves one vital question unanswered – have we made a difference? I will show what you can do to detect improvement and decide if your strategy for quality improvement is successful. Finally, you know what quality you want to measure, you know how you are going to measure it – but how do you carry out the plan? I will finish the presentation with some thoughts on the practical challenges I have had to overcome in running a national clinical audit programme for over 16 years!

Blood components and supply management

2D-S07-01
RED CELL APHERESIS: PROS AND CONS
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Red blood cell (RBC) apheresis for the collection of blood components is performed either as part of multicomponent donations (single-RBC unit collection) or as double RBC (2RBC) collection, where a RBC amount sufficient for two transfusions is obtained with one procedure.
With the devices currently in use for 2RBC apheresis, RBC volumes of 360 ml, 400 ml or 420 ml are collected. Volume replacement with saline and leucocyte depletion of the RBC product are always performed. Because a specified absolute RBC volume is collected, apheresis units have more consistent haemoglobin levels, RBC mass and haematocrit compared to WB donations. Manufacturing steps and RBC loss are much reduced, as are the costs of routine testing.
2RBC apheresis is used for the targeted collection of needed blood groups (i.e. O negative), of RBC of rare blood type or phenotypically matched, and for autologous donation. Where permitted, 2RBC apheresis donation is advantageous for blood donors with hereditary haemochromatosis.
Transfusing two RBC components from the same donor reduces the exposition to allogeneic RBCs for both chronically transfused patients and those requiring a one-time transfusion, decreasing risks for infections and alloimmunization.
National regulations for 2RBC apheresis vary regarding the allowed RBC collection volume and inter-donation intervals (16–24 weeks), but agree on stricter donor selection criteria. Larger total blood volume and higher haemoglobin levels are required for 2RBC apheresis, principally to avoid iron deficiency anaemia. Large studies showed a lower frequency of vasovagal reactions and citrate toxicity with 2RBC donations compared to WB donation and other apheresis. Particular selection criteria, shorter duration of the procedures (25–35 min), smaller amounts of citrate reinfused and volume replacement explain the low incidence of "classical" AEs. However, significant drop-out and lower return rates of 2RBC donors compared to WB donors were also observed. Possible explanations are a higher incidence of minor reactions (i.e. light dizziness and post-donation fatigue), time issues and a negative effect of the longer inter-donation intervals on donor retention. In particular, the role of iron loss associated with 2RBC donation in this context is relatively unclear.
Automated RBC collections allow maximizing RBC yield/donor, a relevant aspect considering the difficulty of retaining active donors due to demographic and social changes. However, the theoretical advantage of 2RBC apheresis in substantially

increasing RBC procurement was only partially confirmed by experience, with great regional differences. In Europe, where automated RBC collections are very variably performed, the proportion of RBC obtained by apheresis (about 25,800 RBC units) was clearly marginal relative to the total number of collected RBC units (2013 EDQM Report). On the contrary, according to the 2013 AABB Survey, 13.8% of all RBC components (approximately 1.9 million units) were collected by apheresis in AABB member institutions, with trend increasing.
RBC apheresis is safe for the donor, has many practical advantages, and allows greater flexibility in specific situations. Still open issues are the influence of iron loss and minor AEs on donor retention. Possible developments comprise the application of pathogen reduction to RBC apheresis components.

2D-S07-02
DO WE NEED CRYOPRECIPITATE IN THE ERA OF FIBRINOGEN CONCENTRATE AND OTHER SPECIFIC FACTOR REPLACEMENT OPTIONS?
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Cryoprecipitate was first used in the 1940s as a source of factor VIII (FVIII) for the treatment of bleeding in haemophilia A. Although still used in some countries for this purpose, its main use now is as a concentrated source of fibrinogen, commonly administered as part of major haemorrhage therapy for patients with uncontrolled bleeding. Fibrinogen is recognised as the first clotting protein to fall to clinically significant low levels during major haemorrhage and there has been a recent explosion of interest around the potential importance of fibrinogen replacement therapy in this setting. This has led many to question the relative benefits of cryoprecipitate as compared to fibrinogen concentrate. This talk will explore whether cryoprecipitate, in the era of specific factor concentrates, is an outmoded treatment.
Cryoprecipitate is manufactured from frozen plasma after controlled thawing. It is not only a rich source of FVIII and fibrinogen but also contains von Willebrand Factor (VWF), FXIII and fibronectin. In UK, cryoprecipitate is available as single donor units or as pools of 5 units and requires thawing before transfusion. Variability in clotting factor levels in blood donors means that the fibrinogen concentration in cryoprecipitate may vary between 3–30 g/l. With the exception of donor screening and viral testing at the time of donation, no viral reduction steps are taken during manufacture.
Fibrinogen concentrate is an alternative concentrated source of fibrinogen, but is not licensed in every country for acquired bleeding. It is a plasma derived product manufactured from pooled plasma. Many view fibrinogen concentrate as a superior product to cryoprecipitate with reasons including: standardisation of production - vials contain a known fibrinogen concentration; lyophilisation making it easily portable and not requiring storage or thawing in blood bank; viral inactivation; and a consideration for the UK is a likely lower risk for vCJD transmission. Gram for gram, fibrinogen concentrate is four times the cost of cryoprecipitate. In UK the haemophilia doctors organisation (UKHCDO) recommend the use of single factor agents above pooled blood components for replacement therapy in inherited bleeding conditions, where possible, for many of these reasons.
But what about cryoprecipitate as therapy for acquired bleeding where patients develop a complex coagulopathy affecting multiple coagulation and fibrinolytic factors? Might cryoprecipitate therapy confer an advantage? Theoretically, higher concentrations of VWF, FVIII and FXIII might promote more rapid primary and secondary haemostasis. Clinical efficacy for the two products in observational studies and in 1 RCT has been reported to be similar, where rises in fibrinogen blood levels are comparable. However, no study has yet been large enough to look at comparative clinical efficacy or indeed safety.
In the era of single factor concentrates, cryoprecipitate may seem outdated, but at present – in particular in relation to therapy for major haemorrhage - insufficient evidence is available which confirms or refutes it place in major haemorrhage therapy.

2D-S07-03
AUDITING AS A MEANS OF DETECTING WASTE
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There can be numerous sources of waste in the transfusion service. The most obvious source is wastage of the blood products themselves, either by expiration on the shelf of the blood bank, or else after they have been issued to a patient location in the hospital but not used and not returned to the blood bank in time to be returned into the general inventory. Reducing expiration due to the former mechanism is the sole responsibility of the transfusion service - to make sure that its inventory is commensurate with the volume of transfusions performed at the hospital(s) that it serves. Reducing the waste of blood products after they have been issued can be a complex process given the multitude of locations where blood is sent in the hospital, and the number of clinical services (and clinicians) that use the products. There are other less obvious sources of wastage in the transfusion service and they can include performing unnecessary patient testing, and using protocols that waste the technologists' time. Audits of clinical practice, and the practices in the transfusion service itself, are essential to identify if these sources of waste are occurring. For example, evaluating the crossmatch:transfusion (C:T) ratio for the various clinical services can identify those that order far more crossmatches compared to the number of red blood cell (RBC) units that they transfuse. Addressing and correcting this issue with the services with high C:T ratios can reduce the workload on the transfusion service by when fewer crossmatches are ordered, and the RBC inventory can be more easily managed if fewer RBCs are reserved for specific patients. Auditing the transfusion service can reveal wasteful practices, too. For example, the crossmatch:issue (C:I) ratio can indicate if the transfusion service is optimizing their process (i.e., method and timing) of performing RBC crossmatches. This review will discuss different types of audits that can be performed in and out of the transfusion service with a view to detecting wasteful practices, and will discuss some practical solutions to common wastage problems.

Cellular therapy

2D-08-01
THE ROLE OF THE TRANSFUSION PHYSICIAN IN SUPPORTING CELLULAR THERAPIES
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The transfusion physician (TP) has several roles in supporting cellular therapies. Let's start analyzing the first cellular therapy that was available and is still the most performed, the hematopoietic cell transplantation (HCT). The participation of the transfusion physician, started already with the performance of the first HCT when bone marrow was used as a source of hematopoietic progenitor cells (HPC), assuring the obliged transfusion support during the aplasia period with all the modifications of the blood components needed to assure the success of the HCT, from the gamma irradiation of the cellular blood components, to the selection of CMV negative/leukoreduced products when the patient required that. Another important intervention of the TP in case of ABO/RhD mismatched HCT was the selection of ABO/RhD groups of the blood components to transfuse to the patients during the different phases of the transplant. The development of colony stimulating growth factors such as granulocyte colony-stimulating factors (G-CSF) for HPC mobilization from bone marrow to peripheral blood, opened the door to the collection of HPC using apheresis separators first to patients and finally also to healthy donors after confirming the long term safety of the mobilizing drugs. The selection of donors, the mobilization treatment and the apheresis collections of HPC increased significantly the task of the TP in the HCT process.
In recent years other treatments have increase even further the functions of TP. The introduction of the extracorporeal chemotherapy (ECP) in the treatment of the acute and chronic graft vs host disease (GVHD) is one example of new treatment. The demonstration of the safety and efficacy of ECP for treating GVHD has made that the number of treatments administered is increasing significantly. In ECP the role of the TP is particularly relevant when the off-line modality of treatment is performed which combine the collection of mononuclear cell (MNC) using an apheresis separator and, after the cells are illuminated with ultraviolet light using an external source. Another form of treatment of GVHD where the role of TP is essential is the preparation of different forms of eye-drop for treating refractory ocular GVHD. Autologous serum first and more recently autologous platelet lysate have shown the efficacy and safety of these rich platelet growth factor preparations in the treatment of ocular GVHD.
The experience generated in MNC collection has been used for introducing new forms of cellular therapies. For instance the MNC product collected by apheresis has been used as starting material for preparing different forms of dendritic cell

immunotherapy. More recently MNC collected by apheresis have been used for producing chimeric antigen receptors in T cells for treating several forms of cancer.
In summary, the TP has been an essential part of the team performing cellular therapies from the beginning of the introduction of the treatment. New developments in the field have increased even further the role of the TP in this form of therapies.

2D-08-02

HAEMATOPOIETIC STEM CELL TRANSPLANTATION FOR ADULTS WITH NON-MALIGNANT DISEASE

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Stem cell transplantation for non-malignant conditions is an evolving field in the adult and older population. This session aims to discuss important principles in this area with reference to three main disease groups; aplastic anaemia, sickle cell disease and transplantation for autoimmune disease. Compared to transplantation for malignant disease there are important points of difference when treating a non-malignant condition. Issues such as minimizing graft vs host disease (GVHD) for which there is no advantage in non-malignant haematological conditions will be discussed with reference to evolving protocols for aplastic anaemia and sickle cell disease.
Stem cell transplantation for adults with aplastic anaemia is an established curative procedure. It is recommended as upfront therapy or after failure of immunosuppressive agents depending on factors such as disease severity and donor availability (whether matched sibling or unrelated). Whilst an established evidence base and outcome data exists for younger adults, data in the older population where unique challenges such as increased co-morbidities and poorer performance status may exist are limited. In the context of an aging population international registry data indicates increasing numbers of transplants are being performed in older patients. In addition to current outcomes for younger patients this session will review important factors of relevance when proposing transplantation for the older population with aplastic anaemia. The role of transplantation in comparison to other therapies such as standard immunosuppression and emerging treatments such as eltrombopag will be discussed. The importance of distinguishing aplastic anaemia from overlapping conditions such as hypoplastic myelodysplastic syndromes will be reviewed. Issues such as chronological vs biological age must be considered and a thorough evaluation of co-morbidities performed in order to assess the suitability of older patients for transplantation. With regard to sickle cell disease, whilst this is an established modality of treatment for children transplantation for adults with this condition has been limited by the absence of suitable protocols for adults and concerns regarding transplantation in the context of increasing co-morbidities and organ dysfunction as these patients reach adulthood. The advent of reduced intensity protocols that minimize GVHD has resulted in a paradigm shift such that adult patients with severe sickle cell disease can safely be offered sibling donor transplantation. Recent evidence in this area as well as the role of unrelated and alternate donors will be presented. Finally severe autoimmune disease refractory to other therapy may be responsive to high dose chemotherapy and autologous stem cell transplantation. Multiple Sclerosis is one such condition with increasing numbers of patients being referred for stem cell transplantation. Updated evidence and the challenges involved when treating this unique patient group will be reviewed.

2D-08-03

WILL WE STILL NEED HEMATOPOIETIC CELL TRANSPLANTATION IN THE ERA OF TARGETED THERAPIES?

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Hematopoietic cell transplantation (HCT) is curative therapy for many hematologic malignancies and non-malignant diseases. The number of autologous and allogeneic transplants continue to increase worldwide due to many advances, including: (i) patients of advanced age and with co-morbidities are now able to undergo HCT due to the use of reduced intensity conditioning regimens; (ii) a suitable donor can be identified for nearly all patients from unrelated, umbilical cord blood and haploidentical donor options; and (iii) novel conditioning regimens have extended the use of HCT to additional non-malignant disorder such as sickle cell disease. However, HCT may be associated with significant morbidity, including organ toxicity, infections, acute and chronic graft-versus-host disease, and a variety of late effects including subsequent neoplasms. In addition, disease relapse remains a major challenge. Targeted therapies have recently emerged in ever-increasing abundance. For several, promising efficacy data have already led to regulatory approval, and in some cases have decreased the need for allogeneic transplant. Examples include brentuximab in Hodgkin lymphoma, and ibrutinib and idelalisib in chronic lymphocytic leukemia. Targeted therapies are also being utilized for relapsed/refractory patients as a "bridge" to transplant, whereas others have been incorporated into conditioning regimens or post-transplant maintenance. Targeted therapies in clinical trial development include small molecule inhibitors, epigenetic modifiers, immune checkpoint inhibitors, monospecific antibodies to a variety of antigens, bispecific antibodies, antibody-drug conjugates, gene therapy, and T- and NK- cellular therapies. Clinical trials are ongoing for patients with a variety of hematologic disorders and stages of disease, and in some situations, have very promising efficacy data. It is currently unclear where targeted therapies will ultimately find their place within the medicine chest of available therapeutic options, but research is quickly moving the field forward and offering hope to many patients. Will targeted therapies be curative? Will targeted therapies complement or replace HCT? What types of health care systems will be needed for delivery of targeted therapies? Here we review the scope of targeted therapies, their interplay with HCT, and gaze into the crystal ball to project what the future may bring in improving patient outcomes.

Parallel sessions

Donors & Donation: Challenges of terrorism and catastrophies, too much or too little blood

3A-S01-01

LESSONS LEARNED FROM PARIS AND NICE

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On November 13th, 2015 between 9:20 pm and 00:20 am, terrorists organized in three different commandos committed several attacks in Saint-Denis and the East Parisian districts. These attacks, claimed by the terrorist organisation Islamic State, constitute the deadliest series of shootings and suicide attacks that France has experienced, with a final death toll reaching 134 and 356 casualties. France was, for the first time, confronted with a case of polymorphic hyper-terrorism with multi-site attacks (such as those that occurred in New York, Madrid in 2004, London in 2005 or Bombay in 2008).The stake for the French Blood Establishment (Etablissement FranŁais du Sang – EFS) was to face the shockwave that propagated throughout the whole establishment from the very minutes following the events (by ensuring delivery of the blood needed for the injured) impacting all the processes and stakeholders involved in the transfusion chain, which were challenged by a massive blood donor influx as soon as November 14th, 2015.This article covers the impacts of the attacks on immunohematology and delivery activities as well as the actions that were implemented (in partnership with the French government crisis management units) to ensure a sufficient blood supply to all the healthcare facilities to where the injured were flocking.Then, the impacts on labile blood component production are set out, within a context where the media were unceasingly issuing urgent calls for blood donations even though the blood stocks were sufficient to face the consequences of the attacks.EFS integrated organization and available stocks allowed meeting blood requirements. This integrated organization, with EFS delivering approximately 80 % of the blood transfused in France, also enabled a strong control of outdating, after unprecedented blood collections during the weeks following November 13th, 2015.Lastly, the article presents EFS experience feedback from the events which helped improve our crisis management pattern with, especially:- Specific steps implemented in the delivery sites located in hospitals that were flooded by a large number of victims, in order to support immunohematology and delivery teams;- An

analysis of the post-attack donor population and of blood donor retention drivers;- A reflection on optimal organizational schemes to face a donation influx;- Actions to leverage the consequences of a "media wave" that could potentially result in useless, excessive blood collections.The feedback on November 13th, 2015 events in Paris as well as on the event that occurred in Nice on July 14th, 2016 allowed to specify EFS criteria for inventory sizing and to strengthen our capacity to face blood demand in a crisis context.

3A-S01-02

LESSONS LEARNED FROM BRUSSELS

V Compernolle

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On March 22-nd 2016, Brussels was confronted with terrorist attacks using explosive devices. Two nail bombs exploded at the airport and one nail bomb exploded at the subway. Thirty-five people were killed, including 3 terrorists, and approximately 340 persons were injured. Patients were transported to several hospitals in the entire country.

Within our blood establishment, stock levels for all blood groups were above 5 days. In Flanders, most hospitals maintain a stock level of 7 days for all blood groups. The number of blood distributed on March 22-nd was – although at the high end – in line with the number of blood products distributed on a regular Tuesday. In contrast, the total number of O RhD negative red cells ordered was higher than average.

We followed during 7 days the number of blood products transfused to victims hospitalized in 3 university hospitals. Overall, the number of blood products needed was limited although a few patients needed high amounts of red blood cells and virus inactivated plasma. The number of transfused blood products was the highest on March 22-nd and decreased during the following days.

Since stock levels were normal we consistently communicated to the media that no additional blood was needed. Even with this message, the number of visits to our website was 4x higher and the number of donors presenting at the donor centers 2x higher than normal. The number of donors presenting at mobile collections on March 22-nd was not increased, mainly due to the fact that one mobile drive had to be cancelled because of the continuing threat. The number of donors on March 23-th was 15% higher at mobile drives and 50% higher the donor centers.

In summary, the experiences in Flanders, following the Brussels attacks, are in line with previous findings in other countries confirming that the amount of additional blood needed is usually limited and the willingness of donors to donate blood is high.

3A-S01-03

CONTINGENCY PLANNING: THE RESULTS TO DATE FROM THE EBA WORKING GROUP

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Background: In 2016 the Executive of the European Blood Alliance (EBA) decided to explore whether there was sufficient interest amongst member countries on whether they saw value in the re-establishment of a Working Group on Contingency Planning. A survey of members was carried out and as there was sufficient interest a Working Group (WG) was established. The question then was how could the work of the WG add value to member countries in their Contingency Planning process. We set out to establish this.

Method: The Executive Director, the EBA office and the Chair of the Working Group sought the views of those members who had been nominated by their BTS on what their expectation from the WG was and also what they saw as tangible deliverables. The result was that there were three areas where the WG felt we should concentrate on and these were (i) Share lessons learned during past contingencies, (ii) exchange contingency plans and (iii) test run contingency plans. As a first step it was decided that we would share our existing Contingency Plans and when these were shared the WG undertook to carry out a gap analysis. It was evident that the state of contingency planning varied across countries. It is vitally important that each BTS ensures that for their critical functions of manufacturing, testing and supply management they have contingency arrangements in place whether that is within the country or with an international partner. But having them in place is not enough they must be tested on a regular basis to ensure that they work. It was important that the WG formulate formal terms of reference to inform its work and also to set out clear deliverables. Draft Terms of Reference (ToR) were drawn up and sent to the Board of the EBA for approval in line with the normal procedure for WGs. These have been approved by the Board and the WG is continuing to develop its work programme to fulfill these ToR.

Outcome: The value that it is intended to deliver is that there is a better understanding of Contingency Planning with tangible examples and case studies which demonstrate how these plans have been activated and the lessons learnt from the events that required their activation. Some of the lessons learnt to date are; (i) that there can never be too much communication on the contingency arrangements and there must be deputies for the main functional leads in the plan, (ii) inevitably the event occurs at a weekend where the nominated people are not always available, (iii) the carrying out of a desktop exercise can be very useful and can expose any shortcomings in the Contingency Plan in a safe environment and (iv) communication between the BTS and their contingency partner must ensure that any changes by either party are communicated so that any impact is worked through. For example Ireland carries out its testing and manufacturing on one site. Ireland has contingency arrangements for testing with Scottish National Blood Transfusion Service which is tested four times per year, three times by air and once by sea to cater for an eventuality such as ash cloud. The contingency arrangement for manufacturing is within the country with a back-up arrangement with NHSBT to import blood if required. Both of these contingency arrangements have been tested. The work of the WG is continuing.

Immunology of Blood Cells: NGS

3A-S02-02

AN ALGORITHM FOR AUTOMATED TYPING OF RED BLOOD CELL AND PLATELET ANTIGENS FROM WHOLE GENOME SEQUENCES

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Background: Humans possess over 300 red blood cell (RBC) antigens along with 33 platelet (PLT) antigens. Exposure to non-self red blood cells (RBCs) and platelet (PLT) antigens during transfusion or pregnancy can lead to the development of alloantibodies, that can cause clinically significant and even fatal complications. Therefore, antigen typing of both recipient and donor RBCs and PLTs is vital and improves transfusion practice and outcomes. Conventional serologic antigen typing is labor intensive, costly, sometimes equivocal, and the reagents needed are not readily available for many antigens. Similarly, most of the available molecular methods evaluate small genetic regions or a limited number of single nucleotide (nt) polymorphisms (SNPs) making typing of weak/null changes, complex gene rearrangements, and even common carbohydrate antigens, such as ABO, difficult.

Aims: Next generation sequencing (NGS), especially whole genome sequencing (WGS), has the potential to overcome some of limitation of serology/SNP typing and provide accurate and high resolution typing by sequencing large gene regions with the ability to evaluate many different types of genetic alterations. However, several challenges must be addressed before NGS based antigen typing can reach its full potential. A critical gap is the lack of computerized algorithms capable of robustly performing automated typing of RBC and platelet antigens directly from NGS data. As such, NGS-based antigen typing can currently only be performed by a few subject matter experts. We recently demonstrated that it was possible to manually analyze NGS-based WGS data to type for RBC and PLT antigens in one individual. Here, we report on the development and initial validation of a novel automated algorithm capable of accurately typing RBC and PLT antigens from WGS data.

Methods: WGS and serology/SNP typings were performed on individuals through the MedSeq Project. An automated WGS based antigen typing algorithm was developed for the 45 RBC and 6 PLT antigen genes. The results were compared with conventional serology/SNP typing for 59 of the most commonly typed antigens, including the ABO and RH blood groups. The algorithm went through two versions with 20 learning samples evaluated using a version 1 of the algorithm and then with 80 blinded samples typed using an improved version 2 of the algorithm. All discordant typings were investigated with the aim of improving the algorithm.

Results: Using the version 2 algorithm, 4,720 antigens were typed in 80 WGS cases. After correcting for 4 serologic typing errors, 4,713 (99.9%) of the WGS typings were concordant with serology/SNP typing. A total of 74 individuals were concordant for all 59 antigens, while 5 individuals had a single discordance and one individual had two discordances. The most common reasons for discordance were misalignment and cis-trans haplotype ambiguities. However, manual review of the WGS data indicated how to potentially overcome these discordances in a future version of the algorithm.
Summary/Conclusions: The automated typing algorithm for WGS based RBC and PLT antigen typing was highly accurate. With further confirmation of accuracy, this algorithm could routinely be used at low cost to type RBC and PLT antigens in all individuals undergoing WGS. In addition, the algorithm input can adapt to other types of data such SNP arrays, whole exome sequencing, and targeted NGS.

3A-S02-03

CONCORDANCE BETWEEN GENOTYPE AND BLOOD GROUP PHENOTYPE IN THE INTERVAL COHORT OF 50,000 ENGLISH BLOOD DONORS

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Background: Clinical validation of high throughput DNA-based methods for identification of blood groups has been identified as an important development for blood services because it can augment or replace grouping by haemagglutination (HA). Available genotyping platforms test for only a limited number of blood group alleles and automated translation of results into blood groups is cumbersome. We have shown that blood groups can be inferred with high accuracy from next generation sequencing results (Lane et al., Transfusion, 2016), but similar analysis on the concordance between genotype and phenotype is lacking for results obtained with genome-wide typing arrays.
Aims: To compare HA-ascertained blood group results from accredited laboratories with results obtained by genotyping of the INTERVAL cohort of 50,000 NHSBT donors (Moore et al., Trials, 2014).
Methods: Genotyping was performed on genomic DNA: (i) with the UK Biobank Affymetrix Axiom array, (ii) by Illumina whole exome sequencing (WES) and (iii) by Illumina whole genome sequencing (WGS). The non-imputed Axiom array typing results for 659 blood group probes were categorised into 'to clinical standard' and 'not to clinical standard' by visual inspection of the call plots. Results obtained with probes from the latter category were excluded from further analysis, those remaining were submitted to a modified version of our typing algorithm to determine blood groups. These data were compared with HA-determined blood groups for ABO [A,B, O], RH [D,C,c,E,e,Cw], KEL [K,k,Kp(a),Kp(b)], FY [Fy(a),Fy(b)], JK [Jk(a),Jk(b)], MNS [M,N,S,s] and LU [Lu(a),Lu(b)].
Results: HA-based blood group results were retrieved from NHSBT's information system and genotypes were called for 820,967 DNA variants, including 659 blood group variants. The analysis of common DNA variants underlying blood groups focussed on the ABO, RH, KEL, FY, JK, MNS and LU systems. We observed concordance levels between HA- and DNA-ascertained blood groups ranging from 95.4 to 100%, except for the D, M, and N antigens. The latter three loci lacked adequate coverage with specific probes. Inspection of the ABO results revealed that 91.5% (43/47 discrepants) of the non-concordant results could be explained by the presence of the *ABO* c.802G>A (p.Gly268Arg) variant for which there is no probe on the array. We selected the *SMIM1* c.64_80del variant underlying the Vel antigen as an example of a clinically relevant low-frequency DNA variant. The genotyping results of 43,678 donors showed 42,430 and 3 donors homozygous for the major and minor allele, respectively and the remaining 1,245 donors tested heterozygous. The minor allele frequency of this deletion, 0.014, in the INTERVAL cohort is identical to the one observed by WGS analysis of 8,066 DNA samples from rare disease cases from the NIHR BioResource.
Summary/Conclusions: Altogether we have obtained compelling evidence that blood group antigen phenotypes can be identified with great accuracy using an affordable genome-wide typing array in conjunction with our newly developed typing algorithm. These encouraging results prompted us to improve the 'not to clinical standard' category of variant probes on the array. We will present the above results and also the genotype-phenotype concordance for results obtained with the next version of the array, by WES and by WGS of the INTERVAL samples.

3A-S02-04

UNORTHODOX O ALLELES AMONG AFRICANS: CHARACTERIZATION OF PREDICTED NEW ABO DELETIONS

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Background: ABO and Rh remain the clinically most important blood groups but despite extensive research over many years, significant findings are still being made. Whilst numerous different null alleles of the *ABO* gene have been recorded, 66% of all defined *ABO* alleles in the 1000 Genomes (1000G*) dataset are null alleles based on c.261delG, where the vast majority represent 25 previously described alleles (*ABO*O.01.xx*). Apart from c.802G>A, represented in ~1% of *ABO* alleles in 1000G, mainly as null alleles (*ABO*O.02.xx*), other rare variants listed by ISBT and also known to give rise to group O are absent.
Aims: While analyzing the 1000G dataset, we encountered two previously uncharacterized deletions potentially giving rise to *O* alleles. The purpose of this study was to explore this finding further.
Methods: The *Erythrogene* database [Möller et al. Blood Advances, 2016], complemented with additional bioinformatic tools, was used to analyze 1000G data files with multiple approaches. DNA samples from selected 1000G donors, together with DNA prepared from samples of African blood donors, were investigated by Sanger sequencing and allele-specific PCR to characterize the predicted deletions. Finally, by comparing known intron markers in major ABO alleles with corresponding ones in the deletional allele, a presumed ancestral major allele could be suggested.
Results: In addition to known *ABO* null alleles, deeper analysis of the 1000G dataset indicated the presence of two alterations not matching any allele acknowledged by ISBT. The first is a large deletion of ~5.8 kbp encompassing exons 5-7. In the 1000G data files, this deletion had been called in 20 individuals and all are heterozygous. The deletion was found in 17 of 661 individuals of African origin, yielding an allele frequency of 1.5% in that continental group. This allele was confirmed and its exact deletion point defined, both by bioinformatic analyses as well as by *in vitro* experiments performed on DNA from 4 of the implicated 1000G individuals. Following development of a PCR assay based on the identified breakpoint, screening of African group O blood donors revealed another 3 donors heterozygous for this deletion, which was thereby phenotypically established as an *O* allele. Closer analysis of genetic markers indicated that the deleted allele most likely originated from *ABO*O.01.02*.
The second alteration detected is a small (24-bp) deletion that had been called by the 1000G algorithms in 9 individuals. Unlike for the large deletion, these individuals were spread across 6 populations in 3 continental groups. It also appeared as if this deletion had occurred on at least 3 different allelic backgrounds. By analyzing BAM (raw data) files and also performing *in vitro* testing on DNA from two of the implicated 1000G individuals, we found no evidence for this deletion. Thus, to the best of our knowledge, the small deletion indicated in the 1000G dataset does not exist in real life.
Summary/Conclusions: A previously uncharacterized *ABO* deletion, prevalent in African populations, was mapped in detail and a genotyping detection strategy devised. The false prediction of another "deletional allele" in the 1000G dataset raises concerns over automated calling algorithms and reinforces that NGS data must be interpreted with caution. Such a caveat is of particular importance for detection of structural variants, insertions and deletions.
Next-generation sequencing results from 2,504 unrelated individuals representing 26 populations worldwide.

3A-S02-05

REFERENCE SEQUENCES FOR THE MOST FREQUENT ABO* ALLELES OBSERVED IN THE ZURICH REGION OF SWITZERLAND

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Background: The availability of allele reference sequences is essential for molecular blood group genotyping and sequencing, particularly if based on next-generation sequencing (NGS). Such allele references sequences should meet the following criteria: (i) complete gene sequence, including introns, exons and flanking regions, (ii) fully phased (i.e. solid haplotype), (iii), confirmed phenotype, and (iv) deposited in a public sequence database (e.g. GenBank). For many blood group systems, however, the availability of such population-based allele reference sequences is still limited. One of the main technical challenges is solid haplotype information for the entire gene, which is difficult to obtain with both classical Sanger sequencing and short-read NGS. A prime example is ABO, considered the clinically most relevant blood group system. Only 11 complete human *ABO* sequences are currently deposited on GenBank (accessed March 8th 2017). Some other deposited sequences just lack the large intron 1, but the majority of sequences are restricted to the exons 6 and 7.
Aims: We aimed to generate high-quality, fully phased reference sequences for the most frequent ABO* alleles of the greater Zurich area of Switzerland, and therefore providing a reference tool supporting ABO blood group genotyping and sequencing.
Methods: To circumvent the haplotype phasing issue, we selected samples being putatively homozygous for the *ABO* gene locus for NGS. In brief, our methodological strategy encompassed three main steps. First, we performed data mining on a large ABO genotype dataset (n = 25,200), which had been generated previously using matrix-assisted laser desorption/ionization, time-of-flight mass spectrometry (MALDI-TOF MS). All individuals were serologically ABO-typed blood donors from the Zurich region. Out of this dataset, we selected 378 individuals being homozygous for three causative SNPs designating A (ISBT wild-type, n = 142), B (c.803G>C, n = 73), O1 (c.261delG, n = 142), and O2 (c.802G>A, n = 21) for extended heterozygosity analysis. Second, to detect further potential allelic heterozygosities, we extended genotyping of these samples by testing 12 additional SNPs, including the SNP c.1061delC, which is discriminative for A1 and A2 alleles. Third, we selected 88 samples being homozygous at all genotyped SNPs and amplified the entire *ABO* gene locus with overlapping long-range PCRs. Amplicons were sequenced in paired-end mode on a MiSeq system (Illumina) using a validated pipeline.
Results: Of all 378 individuals with extended ABO SNP genotyping, 258 samples were homozygous at all 15 SNPs ("SNP-homozygous"), while 120 were heterozygous for at least one SNP. Notably, such heterozygous genotypes were exclusively detected among O1 and A alleles, with two different O1 ("O1-h1", "O1-h2") and two predominant A haplotypes (A1 and A2). Among B and O2 haplotypes no polymorphisms were detected. From all 258 SNP-homozygous individuals, we selected 88 for NGS (12 A1A1; 12 A2A2; 12 BB; 20 O1O1-h1; 20 O1O1-h2; 12 O2O2). We obtained sequences of the entire *ABO* gene (~24.6 kb), spanning from the enhancer region (~3.7 kb upstream of exon 1) to the end of exon 7.
Summary/Conclusions: We have generated a collection of high-quality *ABO* sequences for the most frequent Swiss ABO*A1, A2, B, O1, and O2 alleles with confirmed phenotype. These sequences will serve as a valuable reference resource for NGS-based ABO blood group genotyping and sequencing also in other Caucasian populations.

3A-S02-06

A NUMBER OF NOVEL BLOOD GROUP VARIANTS DISCOVERED FROM THE DENISOVAN HUMAN SEQUENCING PROJECT

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Background: 40,000 years ago, three hominid species co-existed in Siberia – Modern humans, Neanderthals and Denisovans. Interbreeding of the various hominid species and their radiation to distant continents transferred specific genetic variations from ancient humans to distinct populations of modern humans (Harmon, K. 2010). Numerous population sequencing projects examining the genetic diversity of ethnic populations have been undertaken, however in-depth studies into sequence variation of red blood cell genes from populations other than African or Caucasian are rarely examined. Additionally, genetic sequencing data for Neanderthal and Denisovan humans has been available for some time, however little is known of the variation in red blood cell genes between modern and ancient humans.
Aims: To determine the extent of genetic sequence variation in red blood cell genes between Denisovan and modern humans and identify Denisovan-specific novel variants.
Methods: The UCSC genome browser was used to compare sequencing data for all 43 genes encoding the 36 blood groups from the human reference genome Hg19 and Denisova assembly. Denisovan variant call files (VCF) were extracted using the UCSC Data Integrator tool and annotated using ANNOVAR.
Variant nucleotide and amino acid positions were compared to current blood group reference alleles using Erythrogene. Positions identified as potentially novel were cross-checked against the human Hg19 reference genome and dbSNP.
Results: A total of 2555 nucleotide variants were identified in over 40 genes, 61 of which were located in exonic or splice regions. 16 novel variants were identified, these included three in *RHD*, two in *KEL* and two in *CR1* (Knops blood group), as well as a novel splice variant in *BCAM* (Lutheran blood group) and an early termination in *FUT2* (Secretor – H system). One novel *RHD* variant results in an Arg7Gly amino acid change at the same residue responsible for the Weak D type 18 allele (Arg7Trp).
Variants in *ABO* suggest that the Denisovan individual was an O type, similar to findings in Neanderthal man (Lalueza-Fox et al. 2008), and was Fy(b+) homozygous. The Denisovan individual is homozygous for the Memphis variant (Lys56Glu) in the Diego blood group/Band 3 – a variant that has been described in both Melanesian and Indonesian populations, proposed to be the ancestral form of Band 3 (Jarolim et al. 1992, Wilder et al. 2009).
Summary/Conclusions: We have identified a number of novel variants in blood group genes from the sequence of a Denisovan individual, including in *RHD* and *KEL* which potentially may have associated with an antigen. A Lutheran splice variant as well as an early termination in *FUT2* suggest that novel isoforms of both of these blood groups existed in the Denisovan species. Of the 61 exonic variants, 45 are found as blood group alleles and population variants in modern humans and differ in their frequency between ethnic populations. This is the first in-depth examination of blood group variants from the Denisovan sequencing project with identification of a number of interesting novel alleles, potentially important in transfusion, which may yet be identified in under-studied modern human populations.

Blood Safety: Hepatitis

3A-S03-01

VALUE OF RETAINING HBSAG DONOR SCREENING WHERE HBV NAT AND/OR ANTI-HBC DONOR SCREENING APPLY

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Hepatitis B virus (HBV) is a human DNA virus with a global distribution. It is estimated that over 240 million people are chronically infected and more than 680,000 die every year from associated complications, including cirrhosis and liver cancer. HBV comprises a lipoprotein envelope which includes the viral protein, hepatitis B surface antigen (HBsAg) and a nucleocapsid which includes the hepatitis B core antigen (HBcAg) and encloses the viral DNA. As HBV infection is characterised by asymptomatic periods of viraemia, during both acute and chronic infection, it can be efficiently transmitted by transfusion.
HBsAg has been the mainstay to identify and exclude donors infected with HBV since the development of the first HBsAg donor screening test in 1971. The implementation of donor HBsAg screening substantially reduced but did not eliminate the risk of transfusion-transmitted HBV (TT-HBV) because HBsAg is a transient marker which appears 4–6 weeks after initial infection (the acute testing window period) and may disappear several months after infection. In the late 1970's it was recognised that transfusion transmission (TT) occurred more commonly from HBsAg negative donations containing detectable antibodies to anti-HBc (anti-HBc). While some countries subsequently implemented universal anti-HBc screening in addition to HBsAg, this was invariably as a surrogate marker for HIV or non-A-non-B hepatitis, rather than to reduce the risk of TT-HBV.
With the development of HBV DNA tests in the late 1990's, it was recognised that these could be applied to donation screening to 'close' the acute window period,

thereby reducing the residual risk of TT-HBV. Their implementation identified a small proportion of HBsAg negative/anti-HBc positive individuals with detectable HBV DNA, a novel pattern of HBV infection termed occult hepatitis B infection (OBI). Lookback studies subsequently established that blood components from donors with OBI can transmit infection, albeit at a lower rate than blood components collected in the acute window period. Countries that had implemented universal anti-HBc screening (e.g. USA) had therefore already addressed the vast majority of the OBI residual risk, but those reliant on HBsAg screening alone had the option of augmenting HBsAg with HBV DNA and/or anti-HBc. In countries with high anti-HBc rates, which would lead to unacceptably high donor deferral, augmenting HBsAg with individual donation HBV NAT has been the preferred strategy.

Some countries have implemented HBsAg, anti-HBc and HBV NAT, which provides the opportunity to determine the proportional HBV risk reduction contribution of each test. In a large study involving 12.8 million American Red Cross donations screened by all three tests, none of the 1368 HBV confirmed positive donations identified were positive for HBsAg only, and the authors concluded that HBsAg showed no blood safety value. Published data from a number of other countries are consistent with this conclusion; similarly failing to identify 'HBsAg only' positive samples among HBV infected donors. Ultimately, HBsAg testing retains value for characterising the HBV infection stage but the accumulating data strongly suggests that HBsAg testing now adds little, if any TT-HBV risk reduction value where sensitive HBV NAT and anti-HBc screening also apply.

3A-S03-02

AN ASSESSMENT OF DIFFERENCES IN COSTS AND HEALTH BENEFITS OF SEROLOGY AND NAT SCREENING OF DONATIONS FOR BLOOD TRANSFUSION IN DIFFERENT WESTERN COUNTRIES

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Background: The cost-utility of safety interventions is becoming increasingly important as a driver of implementation decisions.

Aims: The aim of this study is to compare the cost-utility of different blood screening strategies in various settings, and to analyse the extent and cause of differences in health economic results.

Methods: For eight western countries (Australia, Canada, Denmark, Finland, France, The Netherlands, UK, and USA) data were collected on donor and recipient populations, blood products, screening tests, and on patient treatment practices and costs. An existing ISBT web-tool model was used to assess the cost-utility of various strategies for HIV, HCV and HBV screening.

Results: The cost-utility ratio of serology screening for these eight countries ranges between -11,000 and 92,000 US$ per QALY, and for NAT between -12,000 and 113,000 US$ per QALY when compared to no screening. Combined serology and NAT ranges between 600 and 217,000 US$ per QALY. The incremental cost-utility of NAT after implementation of serology screening ranges from 2,231,000 to 15,778,000 US$ per QALY.

Summary/Conclusions: There are substantial differences in cost-utility ratios between countries for various HIV, HBV, and HCV screening strategies. These differences are primarily caused by differences in costs of screening tests and differences in infection rates in the donor population. Within each country, similar cost-utility ratio results for serology and NAT compared to no screening, coupled with evidence of limited value of serology and NAT together prompts the need for further discussion on the acceptability of parallel testing by serology and NAT.

3A-S03-03

HBV TRANSFUSION-TRANSMISSION DESPITE THE USE OF HIGHLY SENSITIVE HBV NAT

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Background: Advances in viral nucleic acid testing (NAT) over the last decade significantly reduced the risk of transfusion-transmitted hepatitis B virus (HBV). However, a theoretical residual risk remains even with the implementation of highly sensitive individual donation (ID) NAT. Confirmed post-transfusion acute hepatitis B was recently reported in five (R1-1, R1-2, R2-1, R2-2, R3-1) and one (R3-2) recipients of fresh frozen plasmas (FFP) and red cell concentrate (RCC), respectively. Six different donations from three repeat donors (D1, D2 and D3) initially screened HBsAg (ABBOTT PRISM^{R} HBsAg; Abbott Laboratories; LOD = 0.02 IU/ml) and HBV DNA (Procleix-Ultrio^{TM} Plus ID NAT assay; Grifols; LOD = 1.7–3.0 IU/ml) non-reactive but HBcAb positive in complementary testing were suspected.

Aims: Molecular characterization of HBV strains infecting recipients and suspected donors was conducted to document possible transfusion-transmission.

Methods: Following viral particle concentration, the complete HBV genome and partial S and BCP regions were amplified by nested PCR and sequenced. Phylogenetic analysis of the viral sequences was performed.

Results: HBV full genome sequences were obtained for all recipients and were of genotype D2. Partial S sequence obtained for donor D1 was 99.4% homologous to recipients R1-1 and R1-2 sequences. Further lookback investigations identified 18 blood components from eight previous and one consecutive donations from D1. Post-transfusion data available for 12 recipients (4 FFP, 7 RCC, and 1 PC) identified a third recipient presenting markers of recovered HBV infection but no PC or RCC appeared infectious, irrespective of the corresponding FFP being infectious. Similarly, the near complete genome sequence (2,842 nt) of donor D2 was 99.9% identical to R2-1 sequence. Six other FFP recipients transfused with FFP from five previous and one consecutive D2 donations were investigated. Four recipients showed no evidence of HBV post-transfusion infection, recipient R2-2 (x + 1 donation) exhibited serological evidence of recovered post-transfusion infection without detectable DNA, and recipient R2-3 (x-12) HBV sequence shared 99.9% homology with D2 and R2-1 sequences. No genetic data could be obtained for donor D3 despite several attempts. However, recipients R3-1 and R3-2 were shown infected with 99.9% viral strain homology. These two patients were transfused at 1 month interval in two geographically distinct hospitals. No evidence of HBV infection was observed in seven other patients transfused with RCC (n = 3) and PC (n = 4) from D3.

Summary/Conclusions: HBV transfusion-transmission from two anti-HBc only donors was confirmed in four FFP recipients and supported by indirect strong evidence in two FFP recipients and one RCC recipients. Despite sensitive HBsAg and HBV DNA detection, FFP and to a less extent RCC may remain a risk of HBV transmission when anti-HBc is not screened. Anti-HBc testing might be considered although a considerable proportion of non-infectious blood units would be disqualified in medium or high endemic areas. Negative ID NAT and concentrated viral particles to evidence donor HBV DNA suggest that a viral load <1 IU/ml and an infectious dose <200 IU can be infectious. Highly sensitive HBV DNA detection methods and molecular HBV characterization remain necessary to determine the responsibility of transfusion in suspected transmission cases.

3A-S03-04

SEROLOGICAL CHARACTERIZATION AND RELEVANT MUTATION IN S GENE OF OCCULT HEPATITIS B VIRUS INFECTION AMONG BLOOD DONORS IN CHINA

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Background: A considerable amount of research had been conducted with the discovery of hepatitis B infections characterized by the presence of viral genome without detectable HBsAg (Occult Hepatitis; OBI). But the data about OBI in China nationwide are still absent.

Aims: To investigate the serological characterization and relevant mutation in S gene of occult hepatitis B virus infection among blood donors in China.

Methods: We randomly selected 2977 samples which were reported as HBV DNA+/HBsAg- and sent to National Center of Clinical Laboratories (NCCL) for confirmation by blood centers nationwide during years 2010 to 2016 for this study. We confirmed their OBI status using electrochemiluminescence immunoassay (ECLIA) and individual nucleic acid testing (ID-NAT), determined their serological spectrum by ECLIA, sequenced its S region and analyzed the mutations in the main hydrophobic region (MHR) of them.

Results: The results of ECLIA and ID-NAT showed that 6.11% (182/2977) were HBsAg-positive. HBsAg-/HBV DNA- samples accounted for 52.91% (1575/2977). 7.46% (222/2977) were HBsAg-/HBV DNA+ and negative for all the five serological markers (ie, HBsAg, anti-HBs, HBeAg, anti-HBe, anti-HBc), which were possibly in window period. Ultimately, 33.52% (998/2977) HBsAg-/HBV DNA+ samples were

confirmed as OBI by NCCL. The viral loads of the OBIs ranged from unquantifiable amounts to 4610 IU/ml (Median, 20 IU/ml). Of these OBI samples, 35.87% (358/998) were reactive for anti-HBc and anti-HBe. 29.76% (297/998) were reactive for anti-HBc. The serological spectrum of anti-HBs+/anti-HBc+ occurred in 15.23% samples (152/998) and anti-HBs+/anti-HBe+/anti-HBc+ were detected in 9.92% samples (99/998). The remaining samples had other serological spectrums (9.22%). We randomly selected 116 samples from these 998 OBI samples for sequencing analysis. The result showed the genotypes of the 116 OBI samples were 57(49.13%) genotype B, 57 (49.13%) genotype C and 2(1.72%) genotype D. The subtypes were 57(49.13%) Ba, 48(41.38%) C1, 7(6.03%) C2, 1(0.86%) C4, 1(0.86%) C5 and 2(1.72%) D1. The mutations were observed in 107(92.24%) OBIs. There were 386 amino acid mutations in 57 OBI_B, which were significantly higher than the 269 mutations of 57 OBI_C strains. The most common mutations were V168A (21.55%), K122R (18.1%), Q101R (14.66%), T131N (12.93%), M133T (12.93%), E164G (12.93%). Surprisingly, two kind of drug-resistant mutations, sE164D (rtV173L) and sF161H/L (rtI169T), occurred in 4 OBI patients.

Summary/Conclusions: The low confirmation rate of 33.52% suggested the detection capability of OBI closely related to the sensitivity and specificity of the commercial detection assays used. The overwhelming majority of confirmed samples were reactive for anti-HBc (92.59%) and nonreactive for HBeAg (98.20%). The mutations observed in OBI samples possibly had same correlation with the occurrence of OBI.

3A-S03-05

INVESTIGATION OF REPORTS OF POSSIBLE TRANSFUSION-TRANSMITTED HEV INFECTIONS IN ENGLAND 2012–2016

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Background: Cases of hepatitis E (HEV) have been increasing in the UK over recent years, and heightened clinical awareness has led to more reports to the blood service of possible transfusion-transmitted HEV, with requests for investigation.

Aims: To ascertain whether reported cases of HEV infection in blood component recipients can be attributed to blood component transfusion.

Methods: Key clinical and laboratory information on reported cases was collected. Cases were investigated by retrieving the archived samples of the index blood donations and testing the samples for HEV RNA by individual sample testing. All TTIs were confirmed by showing that donor and recipient viruses were identical through sequencing.

Results: Twenty HEV cases were reported for investigation in the 5 year period. One recipient diagnosis was not confirmed; this case was not investigated. Two cases are in progress. Six of 17 completed investigated cases were transfusion-transmitted infection (TTI), and 11 were not. Investigation of other components from the donations concluded to have caused a TTI led to identification of two further infected recipients. One had already been identified through post-liver transplant surveillance, and had commenced treatment, and the other cleared the infection spontaneously. Ten cases, including the two in progress, were recipients transfused over a significant period of time, often with extensive blood component exposure. Two had a low number of exposures in the relevant time period, and six had availability of stored recipient samples which allowed retrospective testing for HEV status to limit the number of units requiring investigation. The two cases currently under investigation had extensive exposure and no such stored samples, increasing the complexity of the investigation. Eight investigated cases involved recipients diagnosed with chronic HEV infection following investigation of persistently abnormal liver function tests; none were associated with an HEV RNA positive blood component in the relevant time period. The number of components investigated in these case ranged between 3 and 53. The remaining nine investigated cases presented with acute (clinical or biochemical) hepatitis and/or fulminant hepatitis. Three of these cases were multi-transfused and immunosuppressed, and six were not. Six of these nine "acute hepatitis" cases were shown to have been transfused with an HEV RNA positive blood component, and only one of these was immunosuppressed/ multi-transfused. The other three cases of acute HEV were concluded not TTI. The number of components investigated in the acute cases ranged between 10 and 129. The cases concluded to be TTI all presented with "acute" hepatitis: biochemical and/or clinical. They involved investigation of 10, 17, 18, 33, 33, and 129 blood components. Only one case of proven TTI involved red cells. The others were FFP (3), cryo, and platelets.

Summary/Conclusions: In this small series, HEV TTI was more likely when the recipient presented with acute hepatitis. Investigation of HEV cases with chronic hepatitis did not uncover any cases of HEV TTI, but these recipients had often been multi-transfused over long periods. Cases of TTI most usually involved plasma-containing blood components.

Clinical: Clinical transfusion 1

3A-S04-01

BLOOD TRANSFUSION: ONE UNIT TOO MUCH OR ONE UNIT TOO FEW – WHICH POSES THE LEAST RISK TO THE PATIENT?

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Blood transfusion: One unit too much or one unit too few – which poses the least risk to the patient?

During the last two decades, numerous important clinical trial results regarding patient outcome using different transfusion triggers for packed red blood cell concentrates (RBC) were published. These high-quality study results compared restrictive and more liberal transfusion triggers in a specific clinical situation and patient group using highly sophisticated methodology like randomisation, prospective study methodology and important endpoints like perioperative mortality.

However, while answering some important questions in this field, there are still even more questions to be answered here. Most of the published study results deal with surgical or intensive care patients, while patients suffering from neurological, haematological or gynaecological diseases were underrepresented or not included into these studies. In addition, some of these studies accepted a run-in phase, in which both, the restrictive as well as the liberal treatment group was allowed to receive RBC when indicated. Further, in some studies, the liberal transfusion trigger was significantly higher than the trigger used today in most countries, therefore not necessarily representing state-of-the-art treatment.

Preoperative anemia seems to be a relevant indicator for a poor perioperative outcome. However, no prospective trial up to now was able to show, whether preoperative anemia causes the poor outcome or is a sole indicator for a detrimental course instead.

The "single unit" policy adopted in several clinics when transfusing RBC is as evidence-based as the old "dual unit" policy, which was not evidence-based at all.

While the ultimate goal for patients' safety is clear, namely to expose the patient to the minimal amount of allogenic blood products necessary for a safe and speedy recovery while at the same moment giving this said patient the relevant amount of autologous blood products to achieve this recovery goal, the details are by far less clear. Not only the correlation of preoperative anemia and poor postoperative outcome needs to be elucidated, but also the best method to correct preoperative anemia. Whether iv iron, erythropoietin receptor stimulating agents (ESA), blood transfusion, a combination of these or "doing nothing at all" will be the most efficient and safe strategy remains to be seen. Transfusion triggers are not sufficiently defined in acutely bleeding patients, in oncology patients undergoing tumour surgery, chemotherapy or radiotherapy as well as in clinically unstable and old patients. In hematology or neurological patients, these evidence-based transfusion triggers are missing, too. In some trauma entities like central nervous trauma situations, there are no modern study results at all.

For other blood components for transfusion like platelet concentrates (PC), therapeutic plasma (TP) and coagulation factors, transfusion trigger studies are scarce. Since clinical hemotherapy uses combinations of the above mentioned blood components, also the combination therapy needs to be elucidated in clinical trials.

3A-S04-02

CLINICAL USE OF IMMUNOGLOBULIN IN NEUROLOGY

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Immunoglobulin (IG) has been used as a treatment for patients with immune-mediated neurological disorders for more than 3 decades. Given in high doses (2 gr per kg body weight) IG is proving to be effective in a growing number of immune-mediated neurological disorders as a disease modifying treatment. However, many treatment studies have been short term only, and for most chronic disorders the

long-term effect of IG remains to be studied. The effect of intravenously administered IG is firmly established in immune-mediated neuropathies such as Guillain-Barré Syndrome (GBS), chronic inflammatory demyelinating polyneuropathy and multifocal motor neuropathy. In GBS only one treatment with IG is evidence based, however, there is an ongoing randomised clinical study evaluating the effect of a second treatment course in severe GBS. There are other disorders with class 1 evidence of an effect of IG including dermatomyositis, stiff person syndrome and as a rescue therapy in patients with worsening myasthenia gravis. In addition, smaller studies with low class evidence have suggested a positive effect of IG in patients with polymyositis, neuromyelitis optica, autoimmune encephalitis, necrotizing autoimmune myositis and reflex sympathetic dystrophy. In many countries there is a widespread off-label use of IG for the treatment of other neurological disorders despite lack of evidence of the effect. Traditionally, IG is given intravenously which enables a fast induction of the effect. In more recent years, in patients with chronic immune mediated neuropathies with needs for maintenance therapy, subcutaneous administration of IG has also been shown to be effective. This route of administration provides some advantages including less side effects and higher quality of life for many patients.

3A-S04-03

RECOMBINANT FC HEXAMERS AS A PROMISING ALTERNATIVE TO INTRAVENOUS IMMUNOGLOBULIN (IVIG) FOR THE TREATMENT OF ANTIBODY-MEDIATED AUTOIMMUNE DISEASES

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Background: Immune thrombocytopenia (ITP) is an autoimmune disease that is characterized by an insufficient number of platelets in the circulation due to decreased platelet production and increased peripheral platelet destruction. IVIg contains polyclonal IgG molecules that are purified from the plasma of thousands of donors and is recognized as a first-line treatment for ITP and for other autoimmune and inflammatory disorders. Despite its unresolved mechanism of action, the Fc portion of IVIg is critical for its activity. Recent data has suggested that recombinant Fc molecules could become an alternative to IVIg for the treatment of the aforementioned conditions. It was reported that such multimeric Fcs bind Fc gamma receptors (FcgRs) with high affinity. Two different recombinant IgG1-derived Fc hexameric proteins (rFcs) were engineered by CSL and characterized *in vitro* and *in vivo*.
Aims: Our specific aims are to: (1) determine the binding affinity/avidity of the rFcs to various FcγRs in comparison to IVIg; (2) examine the ability of the rFcs to inhibit phagocytosis *in vitro* in comparison to IVIg; and (3) evaluate the therapeutic efficacy of the rFcs compared to IVIg in experimental ITP using a mouse model.
Methods: Recombinant human IgG1 Fc was multimerized by fusing the IgM tailpiece to the C-terminus of either a wildtype human IgG1 Fc (Fc-mTP) or a variant with a point mutation at position 309 (Fc-mTP-L309C). Both rFcs multimerize into hexamers with the Fc-mTP-L309C variant providing a more stable structure in comparison to the wildtype Fc-mTP. Using a human and mouse monocyte monolayer assay (MMA) with human PBMCs and mouse RAW264.7 macrophages, respectively, the ability of the rFcs to inhibit phagocytosis *in vitro* in comparison to IVIg was tested. For ITP, the passive escalating-dose anti-platelet antibody mouse model was used to test the ability of the rFcs to raise platelet counts *in vivo* in comparison to IVIg.
Results: The rFcs bound FcgRs with high avidity and bound to primary cells expressing FcγRs more strongly than IVIg. In accordance with the strong FcgR interaction, the rFcs were exceptional inhibitors of phagocytosis in the MMA, able to inhibit phagocytosis at approximately 375- to 3600-fold lower concentrations than IVIg. The IC_{50} of the rFcs were approximately 8 ng/ml (human MMA) and 413 ng/ml (mouse MMA) in comparison to the IC_{50} of IVIg at approximately 3,000 ng/ml (human MMA) and 1,500,000 ng/ml (mouse MMA). In Balb/c or C57BL/6 mice with ITP, the rFcs were able to raise platelet counts to a higher degree than IVIg at 50- to 400-fold lower doses.
Summary/Conclusions: Our data demonstrates a potent therapeutic effect of the rFcs in experimental ITP, likely due to the blockade of FcgRs triggered by the increased binding avidity to these receptors. These properties make rFcs promising candidates for the treatment of antibody-mediated autoimmune diseases.

3A-S04-04

PATIENT ID – THE CONSEQUENCES OF GETTING IT WRONG ARE SERIOUS

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Background: In Australia, the Serious Transfusion Incident Report (STIR) program was established in 2006 as the Victorian hemovigilance program. Since that time the STIR program has expanded and now receives reports of incidents and reactions from four states and territories. Data on procedural incidents, including near miss events have always been collected.
Aims: To better understand common causes of procedural errors in transfusion.
Methods: STIR has developed investigation forms for both clinical and procedural incidents. Procedural incidents include incorrect blood component transfused (IBCT), wrong blood in tube (WBIT) and near miss events. More recently incidents relating to RhD immunoglobulin have been added. Health services complete an initial on-line notification, after which the STIR secretariat send the specific investigation form for completion. On return, the form is reviewed for validation by one or more members of the STIR expert group for classification, severity and imputability assignment.
Results: Procedural incident data reported here is from fiscal years 2011 until 2016. Annually, between 37–50% of the total STIR reports relate to procedural errors. These include WBIT (n = 318, 68%), IBCT (n = 58, 12%) and near miss (n = 75, 16%). RhD immunoglobulin incidents comprise only a small number of these reports (n = 16, 4%) having only been reportable for just over 12 months.
Approximately 83% of WBIT, 36% of IBCT, 31% of RhD immunoglobulin and 16% of near misses events are related to, or include errors due to poor patient identification. The WBIT, IBCT and near miss forms all include a question regarding the collector having any documented training in blood collection. Close to 50% reported that the collector had received at least one form of training, with hospital induction program being most reported (60%), followed by BloodSafe eLearning (36%), hospital learning package (30%) and in-services (22%). Another 47% of staff involved in procedural errors were reported with unknown training status. Health services have attempted to reduce the incidence of procedural errors, especially in relation to WBIT. A number of health services have instigated staff reflection tools, but the efficacy of such tools is unknown. Other health services have reported the introduction of electronic devices in order to assist the staff member to accurately identify the patient.
RhD immunoglobulin incidents reported also demonstrate patient identification as an issue, with a number of incidents where the product was administered to someone other than the named woman.
Summary/Conclusions: Hemovigilance reporting focuses on all areas of transfusion. Clinical reactions to blood products occur regularly and there has been considerable work to improve the safety of blood. Procedural errors, which occur due to human error, continue to pose a significant risk to patients. Fortunately, most cause no harm, but nonetheless the risk of major morbidity or death remains. Ongoing work required to improve the prescribing, dispensing and administration of blood products continues in health services. A focus on patient identification is required to address many of the issues in relation to blood administration.

Blood products: Transfusion Technology

3A-S05-01

PATHOGEN INACTIVATION OF PLATELETS: IMPACT OF HIGH PLATELET CONCENTRATION AND INTERRUPTION TO AGITATION

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Background: Before a decision to implement a process such as pathogen inactivation (PI) of platelet concentrates (PC) in a large blood service with an extended network of collection and processing sites, it is essential to consider possible variations in the collection/storage of PC that may occur in routine use. In the UK, current

guidance (for non-PI PC) states that interruption to agitation of apheresis platelets during storage must be for "no longer than a total of 24 hours and no single interruption to last for more than 8 hours", in order to maintain pH > 6.4 at end of storage. We investigated whether the same criteria are suitable for PI PC.

Aims: We studied the effect of the INTERCEPT™ Blood System (Cerus), on apheresis PC in plasma, considering two types of variation in collection/handling that might affect platelet quality at end of storage; 1) average or high platelet concentration in PC and; 2) for PC at each concentration, adding periods of interruption to agitation that may occur under normal or 'extended transport' conditions.

Methods: Apheresis PC were pooled and split to achieve four normal concentration (mean 1412 × 10^9/L) double dose PC and four high concentration (mean 1660 × 10^9/L) double dose PC. Two normal PC acted as non treated controls – one of which was handled to mimic extended transport and the other normal transport. Extended transport consisted of three 8 hour interruptions in agitation, whereas normal conditions consisted of four 2 hour interruptions. The second two normal PC were INTERCEPT treated and, again, one was handled so as to mimic extended transport and the other normal transport. These treatments were replicated with the four high concentration PC. All PC were stored agitated at 22 ± 2°C and were sampled and tested on day 2,4,7 and 8 of storage. This was repeated six times for each arm of the study.

Results: In non PI treated PC, no differences in quality were observed between normal and high concentration PC under normal transport conditions. However with extended transport, pH is significantly lower in high concentration versus normal concentration PC, whereas the pH of all units remained >6.4 at day 7. After PI, PC at normal concentration with either normal or extended transport maintained in vitro quality whereas PC at high concentration with either normal or extended transport gave lower pH, supernatant glucose, HSR and ATP levels, whilst CD62P expression and supernatant lactate levels were higher than those at normal concentration. At day 7, 50% of the PI treated high concentration units had a pH of <6.4 regardless of transport conditions.

Summary/Conclusions: This study shows that extended transport can reduce the quality of apheresis PC in plasma, particularly at high platelet concentration, but that current guidelines remain appropriate. This effect was exacerbated by PI treatment. These data suggest that it may be necessary to limit the concentration or length of time PI PC are not agitated, if storing to day 7. Alternatively, the use of platelet additive solutions could be considered to mitigate this effect.

3A-S05-02

CRYOPRESERVATION OF BUFFY COAT PLATELET CONCENTRATES PHOTOCHEMICALLY TREATED WITH AMOTOSALEN AND UVA LIGHT

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Background: Storage time for platelet concentrates (PC) in most countries is limited to 5 or 7 days. Cryopreservation is considered an encouraging approach for extended platelet (PLT) storage, bridging inventory shortages of conventionally stored PLTs. PLTs cryopreserved (CPPs) in 5% dimethyl sulfoxide (DMSO) are currently in clinical development. Preservation of PLT functions and avoidance of transmission of infectious agents via CPPs remain a challenge. The INTERCEPT™ Blood System (Cerus Europe BV) uses a photochemical treatment (PCT) with amotosalen and UVA light to inactivate pathogens and leukocytes. Consequently, INTERCEPT-treated CPPs may lower the patient safety risks. However, a comprehensive characterization of INTERCEPT-treated CPP is essential prior to clinical studies.

Aims: The objective of this study was to analyze potential effects of the INTERCEPT treatment on CPPs as compared to untreated CPPs. Functional, phenotypic and apoptotic properties of such PLTs were analyzed.

Methods: Eight buffy-coat (BC)-PLT units were PCT treated (Test) and eight untreated BC-PLT units served as controls. Test units were exposed to 3 J/cm^2 UVA light in the presence of 150 μM amotosalen. Unbound amotosalen was removed by adsorption in the CAD (Compound Adsorption Device) during 15 h under agitation. A volume of 25% DMSO in 0.9% NaCl was added to the test and control PLT units to achieve a final concentration of 5% DMSO, transferred into a 300 ml DEHP plastic bag and centrifuged at 1200×g for 10 min. All supernatant solution was removed. The DEHP-bags containing approximately 10 ml CPPs were frozen in flat stainless steel containers with two necks at −80°C for approximately 1 month. The CPPs were thawed in a thawing bath maintained at 37°C for 3 min and then resuspended in 200 ml plasma. Functional, phenotypic properties as well as degree of apoptosis and reactive oxygen species (ROS) activity of all CPPs were analyzed pre-freezing, on day 0 (post freezing and thawing) and day 1.

Results: After thawing, all CPPs show a number of biochemical and ultra-structural changes as compared to the pre-freezing data. Interestingly, no significant differences were observed in PLT content post-thaw (202 ± 31 vs 172 ± 38 × 10^9/unit) and LDH activity (26.5 ± 3.6 vs 27.4 ± 67.0% total) between control and test CPPs. Freeze–thaw recovery was (73 ± 0.1% vs 66 ± 0.1%). Similarly, no significant differences were observed after thawing in the percentage of cells showing apoptotic properties (JC-1 59.3 ± 6.2 vs 63.9 ± 2.7%), surface expression of P-Selectin (68.2 ± 5.8 vs 64.5 ± 4.0), Gp1bα and PECAM-1 comparing test and control. Although differences in the energy generation (ATP) and ROS activity were observed, ADP, Collagen and Thrombin-induced PAC-1 expression remained consistent after thawing between groups. Respectively, control and test CPPs show no major differences during clot formation (ROTEM MCF 71.5 ± 2.7 vs 80.8 ± 0.5). Sustained aggregates were observed in the control CPPs after thawing only.

Summary/Conclusions: Overall, CPPs show a number of ultra-structural rearrangements questioning their functional integrity. However, our data indicate that PCT-CPPs exert hemostatic potential in vitro, not different to untreated CPPs. Therefore, the use of PCT is feasible and may prevent CPPs from being a potential source of infection.

3A-S05-03

PATHOGEN INACTIVATED WHOLE BLOOD: SUPPLEMENTATION WITH FIBRINOGEN PARTIALLY CORRECTS TREATMENT DAMAGE IN A ROTEM MODEL OF MASSIVE TRANSFUSION

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Background: Pathogen inactivation (PI) of blood products reduces pathogen risk but also reduces product quality and/or circulation time. The use of PI treated products in massive transfusion protocols has raised concerns about reduced efficacy of transfusions as the volume of transfusion products used may be much greater than in other transfusion settings. Whole blood (WB) transfusion is increasingly used in both military and civilian medicine for the treatment of massively hemorrhaging patients. The impact of PI on WB transfusions in trauma is unknown. Using an in vitro simulation of transfusion assessed by ROTEM, we reported decreased performance of PI platelets and plasma. Increasing use of WB for trauma patients and the near availability of PI for WB create a need for studies of PI impact on transfusion efficacy in this setting.

Aims: This study aimed (1) to assess the effect of riboflavin/UV based PI treatment (Mirasol, TerumoBCT) on the hemostatic potential in an in vitro trauma transfusion model using rotational thromboelastometry (ROTEM) and (2) to determine whether product performance after PI could be normalized by supplementation with fibrinogen.

Methods: The in vitro trauma transfusion model uses hemodiluted fresh whole blood (representing the hemorrhaging patient) mixed with blood product at ratios typical of physiological conditions during transfusion. To prepare transfusion products, paired ABO-matched WB units at day 1 post-donation were pooled and split. One WB unit was treated with Mirasol while the other unit was left untreated. ABO-matched hemodiluted blood samples (20% Hct) were reconstituted with WB transfusion products as follows: (a) hemodiluted WB with untreated WB product, and (b) hemodiluted WB with PI-treated WB units at different final hematocrit ratios ranging from 27 to 37%. To enhance clot firmness, RiaSTAP fibrinogen concentrate (CSL Behring) at a final concentration of 1 μg/μl was added into all test scenarios and compared to the absence of supplemental fibrinogen. The MCF parameter in ROTEM was used to monitor overall clot strength and reflects fibrinogen efficacy.

Results: Hemodiluted blood replacement with Mirasol-treated WB had a significant decrease in the hemostatic activity compared to replacement with untreated WB. The addition of fibrinogen concentrate resulted in significant improvement of clot strength, reported as delta MCF between the supplemented and control groups, with or without PI treatment ($P < 0.01$). The overall response to RiaSTAP supplementation in the presence of PI treatment was a decrease in the difference between the MCF of PI-treated and non-treated WB from 6.8 ± 0.5 mm to 1.4 ± 0.5 mm, showing significant correction of the PI-induced loss of clot strength ($P < 0.01$). All hematocrit levels modeled showed a similar improvement in clot strength from fibrinogen supplementation.

Summary/Conclusions: In a model of trauma transfusion using Mirasol-treated WB to support hemostasis, we have confirmed that treated WB has reduced clot strength and demonstrated that this can be corrected with fibrinogen. Anticipated negative effects of Mirasol treatment on transfusion efficacy in trauma might be ameliorated by fibrinogen supplementation at the time of transfusion. This interpretation requires assessment in clinical studies.

3A-S05-04

POTASSIUM LEAKAGE AND MEASURES OF THE RED CELL STORAGE LESION IN DONATIONS FROM INDIVIDUALS WITH FAMILIAL PSEUDOHYPERKALAEMIA

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Background: Familial pseudohyperkalaemia (FP) is a rare asymptomatic condition characterised by an increased rate of leakage of potassium ions (K^+) from red cells at refrigerated temperatures. FP affects about 1:400 of the UK population and is usually caused by the minor allele of the non-synonymous SNP (rs148211042; R723Q) in the transporter gene *ABCB6* (ATP-binding cassette, subfamily B, member 6). Through screening of the National Institute for Health Research Cambridge BioResource, and investigation of donations with unusually high potassium levels, we identified 18 individuals with this SNP who were available for recall.
Aims: 1) Identify the rate of potassium accumulation over 35 days of storage in Red Cell Concentrates (RCC) from individuals with FP, prepared similarly to standard blood donations. 2) Assess whether other red cell storage parameters are also altered in FP.
Methods: Blood was collected according to standard practice from 6 of the 18 individuals with FP and 11 gender and age-matched controls without FP who consented for the study, held overnight at 18 −24°C, leucocyte-depleted and processed into RCC in SAGM. RCC were stored at 2–6°C and sampled on days 1, 3, 5, 7, 14, 21, 28 and 35 of storage. Samples were tested for full blood count, red cell microvesicles, deformability, haemolysis, extracellular potassium, pH, glucose, lactate, ATP and 2,3-DPG.
Results: FP RCC had significantly higher supernatant K^+ levels than controls over storage ($P < 0.001$). The initial rate of K^+ release was higher in the FP RCC as K^+ reached near maximal levels of 52.3 [29.7 – 63.0] mmol/l (mean [range]) by day 7, increasing to 68.9 [58.8 – 73.7] mmol/l at day 35. This compared to levels in controls of 15.7 [12.9 – 18.7] mmol/l on day 7 which steadily increased to 48.5 [43.2 – 54.3] mmol/l by day 35. On day 35, all RCC were within reference data of K^+ levels observed in random donor RCC (not genotyped) collected as part of previous laboratory studies (43.7 [16.8 – 79.5] mmol/l; 97.5 percentile 61.4; n = 183).
The red cells from FP RCC had significantly higher MCV than control RCC later in storage (day 21 onwards; $P = 0.032$) and were less deformable ($P = 0.021$) when subjected to high shear stress (30 Pa), but not at low shear stress (3 Pa). No other parameters studied differed significantly between FP and control units.
Summary/Conclusions: We confirmed that the rate of leakage of K^+ is much higher than controls during early storage of RCC from most individuals with FP. These data suggest that if the donor has FP, restricting the shelf-life of RCC to 5 days may not be sufficient to reduce the risk of hyperkalaemia in clinical scenarios such as neonatal large volume transfusion. However, levels of supernatant K+ at end of storage in RCC from FP donors do not exceed the upper limit of those measured in RCC from unselected donors. This is therefore likely to be reassuring for other clinical situations where older red cells are acceptable.

3A-S05-05

DEFORMABILITY OF TRANSFUSED RED BLOOD CELLS IS A KEY DETERMINANT OF TRANSFUSION-INDUCED INCREASE IN HEMOGLOBIN: A STUDY WITH **B**-THALASSEMIA MAJOR PATIENTS

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Background: There is a growing number of studies reporting negative transfusion outcome. While some attributed that to storage-induced lesion to packed red blood cells (PRBC), differing results have been reported by others. However, the focus on the PRBC storage duration as the sole quality measure used in current blood banking ignores the functionality of the transfused PRBC, namely their potential to affect the transfusion effectiveness. To address the role of PRBC functionality in transfusion outcome, we have examined the effect of the PRBC deformability, on transfusion outcome. In a recent study, we demonstrated that the transfusion-induced change in the recipients skin blood flow is strongly dependent on the deformability of the transfused PRBC [Barshtein *et al., Microcirculation* 23:479–486, 2016].
Aims: Since the primary goal of blood transfusion is the elevation of the recipients' hemoglobin (Hb) level, in the presents study we have explored the effect of transfused PRBC deformability on the recipients' Hb increment.
Methods: Patients: To this end, we have employed β-thalassemia Major (TM) patients, who suffer from chronic anemia due to congenital hemoglobinopathy, and are routinely treated with life-long transfusion of packed red blood cells (PRBC) every 2–4 weeks. This forms a relatively homogenous patient's population, and enables monitoring the transfusion outcome through consecutive transfusions in the same patients. Thirty nine splenectomized TM patients of the Hadassah Hospital Thalassemia Clinic were recruited (under Hadassah-Hospital Ethics Committee (#0568-12-HMO)); among them 9 patients who received 4 consecutive transfusions.
Study design: The transfusion-induced Hb increment was determined (using the routine blood bank procedure) by the difference in the recipients' Hb level prior to transfusion and 10 min after completion of transfusion. The PRBC were subjected to determination of RBC deformability, using the computerized Cell-Flow-Properties-Analyzer (CFA) designed and developed in our laboratory. The RBC deformability is expressed by the shear stress-induced change in the cell elongation ratio ER = *a/b*, where *a* and *b* are the major and minor cell axes, respectively. The CFA image analysis produces the ER distribution in the RBC population, from which a series of deformability parameters are derived.
Results: According to the local blood bank routine, all the PRBC units given to the TM patients were stored for less than 10 days, and subjected to leukoreduction and γ-irradiation. No correlation was observed between the PRBC ER and storage duration, as expected for shortly-stored units. The transfusion-induced increment in the recipients' Hb was lower than the normally expected 1 g/dl/unit. The transfusion-induced Hb increment, as well as the time interval between consecutive transfusions, were clearly elevated with increasing deformability of the transfused PRBC.
Summary/Conclusions: This study provides, for the first time in humans, direct evidence that the deformability of transfused PRBC is a potent effector of transfusion effectiveness. Currently, PRBC are tested exclusively for immunological compatibility, and supplied primarily by the first-in-first-out (FIFO) criteria, while their functionality is ignored. The testing of PRBC hemodynamic quality introduces a new paradigm into blood-banking, which would contribute substantially to improving transfusion therapy, especially for patients who are treated with life-long frequent transfusions.

3A-S05-06

COMPARING THE EFFECT OF CRYOPRESERVATION ON OVERNIGHT HELD BUFFY-COAT DERIVED PLATELET POOLS SUSPENDED IN EITHER PLASMA OR SSP+

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Background: Cryopreservation of platelets has predominantly been performed with apheresis platelets in 100% plasma. Given the move away from apheresis components in the UK and the use of platelet additive solution, combined with the introduction of overnight hold of whole blood donations prior to processing it would be useful to evaluate freezing of platelets produced via the buffy-coat route of production.
Aims: To compare buffy-coat produced platelet units suspended in either plasma or SSP+ cryopreserved and then re-suspended into either 100% fresh plasma or 100% fresh SSP+ solution.
Methods: Day 2 platelet concentrates were prepared from twenty overnight held whole blood units, with 10 being re-suspended in 100% plasma and 10 in approximately 70% SSP+ and 30% plasma. To these units 6% v/v DMSO was added, an empty satellite bag was attached and units centrifuged. Excess plasma/SSP+ was expressed off leaving approximately 20 mL in the original storage bag. These platelet pellets were then re-suspended, wrapped in protective packaging and stored at −80°C.
On thawing, platelets were either re-constituted in fresh plasma or 100% SSP+. Sample analysis was carried out pre cryopreservation, post thaw and post 4 hours. Measurements of functionality, metabolism, morphology, activation and quality were all assessed.

All measurements are reported as means ± standard deviation and any differences $P < 0.05$ were considered significant.
Results: Following cryopreservation, we observed a platelet recovery of 87% for units stored in plasma and 78% in SSP+. Over the course of the study pH remained stable with all values falling comfortably within the UK "red book" guidelines (≥6.4). However we did note a deterioration of platelet parameters post thaw, regardless of media stored and re-suspended into. We observed increases in cell surface phosphatidylserine expression as measured by Annexin V (3.1 ± 1.0–56.4 ± 13.9% for plasma and 3.6 ± 1.3–50.1 ± 1.5% for SSP+). This combined with increases in microparticle concentrations is suggestive of platelet activation, however measurements of CD62p (p selectin) showed no significant changes over storage. We also observed CD42b (GP1bα) levels to decrease in plasma (53.1 ± 10.1–23.5 ± 6.2 MFI) but did not note any significant change when stored in SSP+ (51.1 ± 4.9–53.7 ± 16.0 MFI). Aggregation assays such as the Impact R cone/plate assay, also showed platelets to still be viable.
Summary/Conclusions: Overnight held buffy-coat platelets prepared in SSP+/plasma (65:35) and then re-suspended post thaw into 100% SSP+, can be effectively frozen and retain function comparable to those stored in 100% plasma. Because of safety and standardisation issues with plasma, we suggest that thawing platelets into an additive such as SSP+ may be more beneficial, for cryopreserved platelet units.

Plenary Session: Major Bleeding

PL1-01
DAMAGE CONTROL RESUSCITATION: HOW TO USE BLOOD PRODUCTS AND MANAGE MAJOR BLEEDING IN TRAUMA

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Trauma is the leading cause of death in the world among those younger than 44. Exsanguination comprises 30% to 40% of mortality in trauma and is the leading cause of death in civilian and military populations. Over the years many different strategies have been suggested for the management of hemorrhage, but in the past 3 decades damage control resuscitation (DCR) and damage control surgery (DCS) have become mainstays of treatment. The term damage control comes from the United States Navy. Damage control refers to maintaining a massively damaged ship afloat with the minimal repairs possible until it can be returned to port for formal restoration of function. The main principles of DCR are permissive hypotension as well as prevention of the 'lethal triad' of acidosis, coagulopathy, and hypothermia. Acidosis affects multiple physiologic processes including cardiac contractility as well as worsening coagulopathy. Hypothermia can worsen acidosis and cause coagulopathy. DCR is designed to prevent the lethal triad by minimizing crystalloid administration and providing a balanced blood product resuscitation that is plasma and platelet rich in a 1:1:1 ratio of red cells:plasma:platelets to mimic whole blood. Patients also are aggressively rewarmed. Adjuncts such as cryoprecipitate and tranexamic acid are frequently required and their use may be guided by new coagulation tests including thrombelastography (TEG) and rotational thrombelastometry (TEM).DCS is used to complement DCR. DCS focuses on rapid and immediate repairs including control of hemorrhage, temporary repair of hollow viscus injuries with the gastrointestinal tract left in discontinuity, and packing to control non-surgical bleeding. The patient is then transferred to the intensive care unit (ICU) and undergoes further resuscitation with DCR for treatment of coagulopathies and metabolic derangements. Once the patient is adequately resuscitated they are then taken back to the operating room in the next few days for definitive surgery. DCR and DCS utilization have improved outcomes for trauma patients but further research is needed to continue to decrease preventable mortality from hemorrhage in the severely injured trauma patient.

PL1-02
DIRECT ORAL ANTICOAGULANTS (DOACS) INDUCED BLEEDING – UPDATE ON ANTIDOTES AND TREATMENT OPTIONS

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Specific Antidotes. Three antidotes for the DOACs are under various stages of development. The RE-VERSE trial was a prospective cohort study that showed that idarucizumab administration reversed anticoagulation as evidenced by the normalization of the dilute thrombin time and ecarin clotting time within minutes among subjects suffering a serious hemorrhage or who required an urgent procedure. [1] However, only a preliminary analysis has been published to date, and further analyses will be required to look at the efficacy and safety of idarucizumab in term of bleeding, transfusion, outcomes, and mortality. Andexanet alfa is a recombinant modified human factor Xa protein that serves as a specific reversal agent to neutralize the anticoagulant effects of direct and indirect factor Xa inhibitors. A recent study revealed that andexanet alpha reversed the laboratory assessed anticoagulant activity of rivaroxaban and apixaban in older healthy individuals within minutes of administration. [2] At present, the single arm, open-label trial (ANNEXA-4) is under way to confirm the clinical benefit of this drug in patients on apixaban, rivaroxaban, edoxaban, or enoxaparin who present with an acute major hemorrhage. Non-specific options. Despite the optimal administration of those specific antidotes, patients may continue to bleed because of vascular injury, complex coagulopathies, or other issues associated with critical illness. Adjunctive treatment may be useful, and there may be a role for prothrombin complex concentrate in patients with continued bleeding or in centers without access to antidotes. Studies performed on animal models reported that coagulation factor concentrates could be used for bleeding management, and prothrombin complex concentrates (PCC) administered at doses ranging from 50 to 100 U/kg could normalize coagulation laboratory parameters. [3] Although the results on bleeding endpoints were less consistent, most studies reported a significant decrease in blood loss, or a significant reduction in hematoma's volume after the administration of these agents in animal models. Other studies reported that activated PCC (aPCC) could be considered as a better alternative compared to PCC alone. [4] However, the use of aPCC might be associated with an increased risk of thromboembolic complication, and aPCC might be considered as a rescue therapy in case of life-threatening bleeding. To date, the efficacy and safety of PCCs and/or rFVIIa for the management of bleeding in patients treated with DOACs is limited to studies performed in healthy volunteers. Recommendations on whether coagulation factor concentrates should be part of our management strategies are based on experts' opinions, and further studies are needed before any evidence-based recommendations could be formulated. References1 Pollack C, Reilly P, Eikelboom J, et al.: Idarucizumab for Dabigatran Reversal. N Engl J Med 2015; 373: 511-20.2 Connolly SJ, Milling TJ, Eikelboom JW, et al.: Andexanet Alfa for Acute Major Bleeding Associated with Factor Xa Inhibitors. N Engl J Med 2016.3 da Luz LT, Marchand M, Nascimento B, et al.: Efficacy and safety of the drugs used to reverse direct oral anticoagulants: a systematic review and meta-analysis. Transfusion 2017.4 Martin A, Gouin-Thibault I, Siguret V, et al.: Multimodal assessment of non-specific hemostatic agents for apixaban reversal. J Thromb Haemost 2015; 13: 426-36.

PL1-03
PATHOPYSHIOLOGY OF MASSIVE HAEMORRHAGE

J Stensballe

No Abstract available

Parallel sessions

Immunobiology of blood cells Regulation of carbohydrate blood groups

3B-S06-01

THE P1 HISTO-BLOOD GROUP ANTIGEN IS PRESENT ON HUMAN RED BLOOD CELL GLYCOPROTEINS

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Background: The P1 antigen was first described in 1927 but not categorized with the P^k antigen in the P1PK histo-blood group system until 2010. Individuals of P_1 phenotype have both the P^k and P1 antigens on their red blood cell (RBC) surface, while P_2 individuals lack P1 and have lower amounts of P^k. Like in the ABO system, the antigens are carbohydrate epitopes built up stepwise by glycosyltransferases. The antigens of the P1PK system are synthesized by 4-α-galactosyltransferase (α1,4GalT) encoded by *A4GALT*. Galα4Gal-terminating antigens like P^k and P1 can function as receptors for P-fimbriated *Escherichia coli (E. coli)*. Moreover, the terminal and internal Galα4Galβ sequence is a known Shiga toxin receptor. Antibodies against the antigens of the P1PK system are naturally-occurring. Anti-P1 induced hemolytic transfusion reactions are rare but a few cases have been reported. The anti-$P1PP^k$ found in plasma from individuals of the p ($P1PP^k$—) phenotype is also associated with recurrent miscarriages. In humans, α1,4GalT has been considered to extend glycans on glycosphingolipids exclusively. However, in certain other species, such as the pigeon, the P1 epitope resides on glycoproteins as well. Interestingly, the majority of the human A, B and H antigens are found on glycoproteins and they share the same precursor as P1, namely paragloboside.
Aims: This work is based on a hypothesis stated years ago regarding the P1 glycoepitope. Haselberger *et al.* [FEBS Lett., 1982] published that P1 is carried on glycoproteins in humans. However, this was later contradicted firmly by Yang *et al.* [J Biol Chem., 1994]. The aim of this work was to find out if P1 is present on glycoproteins and if so, to characterize carrier candidates on human RBCs.
Methods: RBCs from peripheral blood of apparently healthy volunteers were used for the study. P1 phenotyping and SNP genotyping was used to determine P^1/P^2 status. RBC glycans were digested with α-galactosidase from green coffee bean, α1,3-specific GH110 α-galactosidase of bacterial origin (B-zyme), papain, neuraminidase and/or peptide-N-glycosidase (PNGase) F. RBCs were lysed and the hemoglobin was washed away while the unsealed membranes were collected. Denatured membrane proteins were separated in SDS-PAGE and transferred to a low fluorescence PVDF membrane, immunoblotted with monoclonal anti-P1 and staining quantified in relation to total protein loaded.
Results: We show clearly that P1 occurs on various glycoproteins, seen as a smear-like pattern in Western blots with cell membranes from P_1 but not P_2 or p RBCs. Furthermore, there was a significant difference between the staining of RBCs from P^1-homozygous (stronger) and P^1-heterozygous (weaker) individuals. The amount detected varied between samples of the same genotype, which is consistent with earlier studies of P1 expression on RBCs. A tendency toward a banding pattern suggested possible carriers at ~50 and ~100 kDa. P1 staining was lost after treatment of the RBCs with coffee bean α-galactosidase as the enzyme destroys the P1 epitope. PNGase F treatment reduced the P1 signal whilst B-zyme did not.
Summary/Conclusions: This study provides data showing that P1 does indeed reside on human RBC glycoproteins and not only on glycosphingolipids. The epitope was detected in a gene-dose dependent manner on glycoproteins of P1-positive RBC membranes. The signal was reduced when using N-glycan-specific PNGase F, indicating that at least some of the epitopes are carried on N-glycans, which mimics the ABH antigens. Attempts to identify specific carrier proteins are in progress.

3B-S06-02

KLF1 REGULATES P1 EXPRESSION THROUGH TRANSCRIPTIONAL CONTROL OF A4GALT

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Background: The P1Pk blood group system includes carbohydrate antigens P1, Pk and NOR present on glycolipids. Specifically, the enzyme A4GALT is responsible for the transfer of galactose to lactosylceramide generating the P1 blood group. Intriguingly, the genetic basis underlying P1 expression is not completely understood. Different SNPs are reported to predict P1 expression, adding to the controversy surrounding this blood group (SNP rs8138197 and the combination SNPs rs5751348 and rs2143918). Another layer of complexity comes with the surprising correlation observed in In(Lu)(Lutheran negative) individuals, which also leads to weakened or negative P1 phenotype. P1 is present on glycolipids which has no known regulatory connection with BCAM, harboring the Lutheran blood group. Interestingly, Lutheran negativity can be caused by mutations in the erythroid specific transcription factor Krüppel like factor 1 (KLF1).
Aims: These data suggest that KLF1 may also be implicated in the regulation of P1, possibly through transcriptional control of A4GALT, a hypothesis that was tested here.
Methods: A cohort of 24 Lutheran negative individuals was analyzed to investigate the regulation of P1 by A4GALT and KLF1. DNA was isolated from PBMC and regions within the A4GALT gene containing SNP rs8138197, rs5751348 and rs2143918 were sequenced. Expression of the P1Pk blood group system and other membrane proteins was evaluated by flow cytometry (e.g. CD44, Lutheran, CD71, Glycophorin A). Overexpression and knockdown experiments were performed by lenti-viral transduction of primary pro-erythroblasts with open reading frames of or short hairpins against KLF1 (or mutants thereof) or A4GALT. Effects on erythropoiesis were studied by flow cytometry, Western blotting and RNA expression analysis.
Results: Sequencing of A4GALT intronic regions that have been correlated with P1-negativity show that SNP's rs5751348 and rs2413918 located between exon 2 and 3 co-segregate with P1 phenotype for 21 out of 24 In(Lu) donors, however all 3 non-correlating individuals harbor KLF1 mutations. SNP rs8138197, previously claimed to explain the P1 phenotype, only correlated for 12 out of 24 In(Lu)s of which 7 may be explained by KLF1 mutations. Knockdown of A4GALT results in complete absence of the P1 blood group in primary erythroblasts, indicating for the first time in primary human erythroblasts the direct link between A4GALT and the P1 blood group. In(Lu) donors with KLF1 mutations have severely reduced P1 expression and RNA-Sequencing revealed significantly lower A4GALT RNA expression, suggesting that A4GALT is a putative KLF1 target gene. KLF1 knockdown during erythropoiesis leads to P1 negativity and re-expression of KLF1 zinc finger mutants does not rescue the P1 phenotype whereas wild type KLF1 does.
Summary/Conclusions: In conclusion, SNP's, rs5751348 and rs2413918 correlate P1 and Pk phenotypes. We found that individuals that contain the P1 positive genotype but harbour a KLF1 mutation, show decreased or negative P1. The data indicates that A4GALT is a KLF1 target gene and that P1 negativity can be caused by KLF1 haplo-insufficiency as result of mutations within KLF1. These results have implications for blood group genotyping as a thorough SNP analysis of the A4GALT gene may not be sufficient to predict P1 blood group phenotype.

3B-S06-03

EX VIVO GLYCOSYLATION OF P2 BLOOD GROUP RED CELLS USING RECOMBINANT P1/PK SYNTHASE GENERATES P1 BLOOD GROUP CELLS

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Background: Human α1,4-galactosyltransferase (Gb3/CD77 synthase, $P1/P^k$ synthase), encoded by *A4GALT*, synthesizes 2 or 3 carbohydrate antigens of the P1PK blood group system depending on the amino acid position 211: presence of Q causes synthesis of P^k and P1 antigens, while E in addition allows synthesis of NOR (Suchanowska, JBC, 2012). P1, P^k and NOR antigens are carried by glycosphingolipids: P1

and P^k terminate with Gal(α1-4)Gal disaccharide, while NOR terminates with Gal(α1-4)GalNAc. P1, P^k and NOR antigens are carried by glycosphingolipids: P1 and P^k terminate with Gal(α1-4)Gal disaccharide and have long been thought to be synthesized by the consensus enzyme (encoded by *A4GALT*), while NOR terminates with Gal(α1-4)GalNAc. The presence or absence of P1 antigen determines the P_1 or P_2 blood group, respectively. Recently, we obtained recombinant forms of the consensus enzyme and its p.Q211E variant, and showed that they can synthesize P^k and P1 antigens or P^k, P1 and NOR antigens, respectively (Kaczmarek, BBRC, 2016).

Aims: To evaluate whether a recombinant catalytic domain of the $P1P^k$ synthase is able to synthesize the P1 antigen on the surface of P_2 red blood cells and 2102Ep embryonal carcinoma cells.

Methods: Papain-treated P_2 blood group red cells and trypsinized 2102Ep cells were incubated in reaction mixtures containing UDP-galactose and recombinant $P1/P^k$ synthase obtained in baculovirus expression vector system. The cells were then analyzed by flow cytometry using human monoclonal anti-P1 antibody (Immucor, clone P3NIL100; recognizes P1 only).

Results: P_2 blood group red cells treated with recombinant $P1/P^k$ synthase behaved like P_1 cells in flow cytometry. The P1 signal strength was dependent on enzyme dose. 2102Ep cells treated with the enzyme displayed the P1 antigen as well, but the signal was weaker than for 2102Ep cells transfected with vector encoding the enzyme.

Summary/Conclusions: We found that recombinant human $P1/P^k$ synthase efficiently transfers galactose residues to the precursor of P1 (paragloboside) on the surface of red blood cells, turning P_2 blood group erythrocytes into P_1 blood group cells. In the same way, 2102Ep cells may be glycoengineered to display P1 antigen on the cell surface. To the best of our knowledge, this is the first report that a recombinant glycosyltransferase may be used to modify carbohydrate moieties of glycosphingolipids residing on the surface of cells. In addition, this is a proof of concept that phenotype of erythrocytes may be changed not only by enzymatic removal, but also by *ex vivo* synthesis of blood group determinants.

3B-S06-04

NOVEL MUTATION OF RUNX1 SITE IN THE ERYTHROID CELL-SPECIFIC REGULATORY ELEMENT EFFECT THE ABO ANTIGEN DIFFERENTIAL EXPRESSION

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Background: The ABO blood group system is important in the context of blood transfusion and organ transplantation. Recently, an erythroid cell-specific regulatory element (+5.8-kb) was identified in the first intron of ABO, which is responsible for the ABO antigen differential expression. It was found that the regulatory activity of the element was dependent upon the mutation of the +5.8-kb region. The individuals with ABO subgroups were always seen in the Chinese population, but there was little information about the function of +5.8-kb region in these individuals in China.

Aims: Study the underlying mechanism of the novel mutation of the +5.8-kb region responsible for reduce of antigen expression in ABO subtypes.

Methods: The nucleotide sequences of the partial intron 1 covering the +5.8-kb region was amplified and directly sequenced. Every mutation on sequences of the gene was analyzed and recorded. The haplotypes with the novel mutation were obtained by the TOPO TA cloning. Both the ABO promoter and the +5.8 kb regulatory element were sub-clone into the basic luciferase reporter plasmid by the double endonuclease cutting. Then the recombinant firefly luciferase report and the pRL-SV40 *Renilla* luciferase reporter vector were transient transfection into K562 cell. Light emission was measured using an absorption spectrophotometer.

Results: A novel nucleotide substitution +5904 C>T located at RUNX1 binding site in the +5.8 kb region was discovered in three individuals with B subtypes. The recombinant firefly luciferase report system was successfully constructed, which carrying the ABO promoter and the mutation +5904 T haplotype in the background of the +5.8 kb site of B. Transit transfection experiments demonstrated that the single point mutation of RUNX1 binding site reduced the activity of the +5.8 kb site. The relative luciferase activity of the mutation+5904 T haplotype was less than about 2 fold of the control with the normal B allele.

Summary/Conclusions: This study suggested that the transcriptional activity of the +5.8 kb site could be down regulated by the single point mutation of RUNX1 motif on the B allele, leading to reduction of B antigen expression.

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3B-S06-05

DOWNREGULATION OF GLYCOSYLTRANSFERASE A EXPRESSION BY MIRNA-331-3P IS MEDIATED BY THE INHIBITION OF THE TRANSCRIPTION FACTOR SP1

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Background: The molecular genetic basis of the ABO system has been known since 1990. More than 100 ABO subgroup-related variations were detected in the coding region of glycosyltransferases, consisting of seven exons, resulting in weak blood group antigen expressions. Beside introns and exons, two regions were found to be critical for transcriptional activity of ABO genes: a promotor sequence upstream of the translation start site and a CBF/NF-Y-binding enhancer element. However, the molecular mechanisms underlying some subgroups could not be explained by current methods.

Aims: By using different approaches, including gene array analysis, luciferase reporter assay and overexpression of glycosyltransferase specific miRNAs in primary hematopoietic stem cells (HSCs), we found that miR-331-3p directly targets glycosyltransferase A and B mRNA. Now we have been further embarking on the underlying mechanisms of miRNA and glycosyltransferase interactions and show that the effects of miR-331-3p are mediated by inhibition of transcription factor SP1, which is a major regulator of the ABO gene, thereby resulting in downregulation of blood group A antigen expression.

Methods: We treated hematopoietic stem cells with different concentrations of a specific inhibitor of SP1 (mithramycine A) and analyzed blood group A expression by flow cytometry, immunofluorescence and ID-Card gel method.

Results: Using microRNA target prediction tools we also identified Sp1 as a potential target gene for miR-331-3p. Western blot analyses of glycosyltransferase protein expression showed that overexpression of miR-331 led to a decreased glycosyltransferase and SP1 protein expression. Further approaches with the SP1 inhibitor, mithramycin A, revealed similar results. Inhibition of SP1 leads to 40–50% reduction of blood group A positive red blood cells (RBC) in A_1O individuals and up to 80% in A_2O individuals. Analysis of $Aw04/O_{1v}$ genotypes revealed elevated miRNA levels, thus confirming microRNAs as regulators of blood group glycosyltransferase expression.

Summary/Conclusions: Our findings extend our understanding of blood group regulation in carriers of weak blood group variants. Involvement of miRNAs in the downregulation of ABO blood group antigen expression may also provide an explanation for observed changes of ABO antigen expression in abnormal processes such as tumorigenesis, pregnancy and aging. Furthermore, this pathway may play a role in the regulation of other blood group variants (for instance Rh, KEL, amongst others).

3B-S06-06

EFFECT OF FUT1 GENE MUTATION ON MRNA AND PROTEIN EXPRESSION IN EUKARYOTIC CELLS

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Background: α-(1, 2) fucosyl -transferase encoded (FUT1) by *FUT1* gene plays an important role in the expression of H antigen on red cells. Mutations in these genes could lead to rare Bombay or para-Bombay phenotypes, which lacked or weaken H antigens expression on their red cells. Five site-mutated FUT1 genes (35C>T, 235G>C, 293C>T, 658C>T, 682A>G) and one deleted mutation (547-552delAG) were identified in our lab. But all the mutations were heterozygous in nine individuals and the influences of all these mutations on mRNA transcription and FUT1 enzymes' activity are unclear.

Aims: Construct recombinant proteins of FUT1 mutated genes to explore the effects of mutations on mRNA transcription, FUT1 enzyme expression and activity in vitro expression system.

Methods: The full coding region of mutated FUT1 genes was amplified and ligated with pcDNA3.1 plasmid to construct the recombinant expression vectors, and then the recombinant plasmids were transfected into COS-7 cells. Transient analysis and stable expression screening were performed. *FUT1* mRNA expression was determined by real-time quantitative PCR (RQ-PCR). The activity of enzyme was measured by high performance liquid chromatography (HPLC). Recombinant protein was

identified by SDS-PAGE and western-blotting and structure modeling of FUT1 protein was constructed by swiss-model software.
Results: ① 35C>T, 235G>C, 293C>T, 658C>T, 682A>G, 547-552delAG mutations were identified in nine individuals with para-Bombay phenotype and the frequency of para-Bombay phenotype was below 1:20000 in blood donors. ② The eukaryotic expression vectors of wild *FUT1* and 35C>T, 235G>C, 293C>T, 658C>T, 682A>G, 547-552delAG, 35C>T + 682A>G and 35C>T + 235G>C + 682A>G mutants were successful constructed and confirmed by cloning and sequencing. The stable expression COS-7 cells were obtained after recombination plasmid transfection and screening with 500ug/ml G418. ③ The mRNA transcription of transfected cells with 35C>T, 235G>C, 293C>T, 658C>T, 682A>G, 547-552delAG, 35C>T + 682A>G and 35C>T + 235G>C + 682A>G were almost at the same level with wild-type FUT1, which reached 99.63%, 103.57%, 97.10%, 104.74%, 101.69%, 102.79%, 100.20% and 100.79% of the wild-type, respectively. ④ A specific protein band about 46kD was confirmed in the wild-type, 35C>T, 235G>C, 293C>T, 658C>T, 682A>G, 547-552delAG, 35C>T + 682A>G, 35C>T + 235G>C + 682A>G transfected cell lysates by SDS-PAGE and western-blotting with 6 × His Tag antibody, but not found in 547-552delAG transfected cells. ⑤ The enzymes activity of cell lysates transfected with 35C>T, 682A>G, 35C>T + 682A>G, 35C>T + 235G>C + 682A>G, 235G>C were 79.45%, 61.06%, 29.34%, 21.41% and 16.23% respectively compared with wild-type *FUT1*, while the enzymes activity of cell lysates transfected with 547-552 deletion AG, 293C>T, 658C>T were abolished. With regard to the Phenyl-galactoside substance, $Km^{phenyl\text{-}gal}$ of the wild transfected cell lysates was 83.6 μmol/l, while $Km^{phenyl\text{-}gal}$ of the C35T transfected cell lysates was 125 μmol/l.
Summary/Conclusions: Our data suggested that the mutations of FUT1 gene did not affect the RNA and protein expression level, but affected the enzymes' activity. The 235G>C and 682A>G mutations of FUT1 gene significantly decreased the encoded FUT1 enzymes' activities. 293C>T, 658C>T, and 547-552delAG mutation abolished enzymes activities which may result in the reduced expression of H antigen and lead to para-Bombay phenotype.

Blood Safety: Retrovirus

3B-S07-01
COMPARISON OF THREE METHODS FOR ESTIMATING RESIDUAL RISK OF TRANSFUSION-TRANSMITTED HIV-1 INFECTION IN FRANCE

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Background: Estimates for HIV-1 residual risk (RR) have relied on a classical method derived from repeat blood donor histories (cohort method). As blood donations found to be HIV-1 antibodies (Ab) positive are further tested to detect Recent Infections (≤180 days) using a detuned assay (EIA-RI), and since HIV-1-RNA Nucleic Acid Testing (NAT) was introduced in 2001, two additional methods estimating HIV-1 incidence are currently available.
Aims: To compare HIV-1 incidences and residual risks obtained with EIA-RI and NAT methods to those derived from the cohort method over 3-year periods between 1992 and 2015.
Methods: With the EIA-RI method, incidence = [recent infection cases/(donations*(180/365)]. With the NAT method, incidence = [(RNA+/Ab- cases)/(donations*(T/365)] where T is the time between RNA detection and Ab detection (12 days). These estimates were compared to those obtained using the cohort method [Seroconversions/Donor-Years (DY)]. Residual risks were calculated with the classical Incidence-Window-Period method.
Results: Between 1992 and 2015, 67 million donations were collected in France (56.5 million in repeat donors and 10.5 million in first-time donors). Among the 338 HIV-1 positive repeat blood donors tested with the EIA-RI, 137 (40.5%) were identified as recently infected. Among the 238 repeat donors found HIV-1 positive since 2001, 18 (7.6%) were HIV-1 RNA+/Ab-. Incidence rates derived from the EIA-RI were similar to those obtained through the cohort method in any of the 8 study periods: incidence decreased from 3 per 100,000 DY in 1992-1994 to 1 per 100,000 DY in 2013-2015. With the NAT method, incidence rates calculated since the 2001-2003 period were not significantly different in any of the 5 study periods (1.3 per 100,000 DY in 2013-2015). HIV RR estimated with the cohort method was 1 in 3,200,000 donations in 2013-2015. On the same period in the overall donor population, HIV RR was estimated to be 1 in 3,300,000 million donations with the EIA-RI method and 1 in 2,850,000 donations with the NAT method.
Summary/Conclusions: This study shows that alternative methods (EIA-RI and NAT) can be used to estimate HIV-1 incidences in a population with low HIV-1 incidences. However, since the time between RNA detection and Ab detection is short (12 days), the NAT method should be used on very large samples, such as the blood donor population, to provide accurate estimates. These two alternative methods are particularly interesting since they can be used to estimate incidence in the overall donor population and not only among repeat donors. The three methods confirm the current extremely low risk of HIV-infected donations (between 1 in 2,850,000 and 1 3,300,000 donations) entering the blood supply in France.

3B-S07-02
RESIDUAL RISK OF HUMAN IMMUNODEFICIENCY VIRUS AMONG BLOOD DONORS IN BOGOTA, COLOMBIA

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Background: The estimation and regular monitoring of the risk that an infection can be transmitted by blood transfusion, despite sensitive screening tests and rigorous selection of donors, is essential to establish the situation and dynamics of blood safety within a region. Furthermore, it makes possible to predict the magnitude of risk reduction that could be achieved if more sensitive screening methods were introduced. To date, there are not published studies on estimates for residual risk of transfusion-transmitted HIV infection in Colombia.
Aims: To estimate the residual risk of HIV infection among blood donors in Bogota, Colombia.
Methods: A database was designed to facilitate the identification of donors and all donations performed from January 2011 to December 2016 at the National Blood Bank Colombian Red Cross in Bogotá, Colombia. Incidence rates were calculated from donors who gave blood at least twice during the study period, considering the full length of the seroconversion interval. The residual risk for repeat donors was calculated by the classic method of incidence rate / window period described by Schreiber et al., (1996). The window period was obtained from literature for chemiluminescence immunoassays in the Abbott Architect platform. To estimate the global incidence rate (for first time and repeat donors), the incidence rate form first time donors reported in Brazil was used, as well as the relative proportion of first time and repeat donors in the studied population. Confidence intervals (CIs) for incidence rate was derived from Poisson regression.
Results: There were 142,804 donations during the study period and 82,688 (70.66%) were from first time donors. Just 24,259 donors gave blood at least twice. Four seroconversions were identified, yielding an incidence rate estimate of 16.77 (95% CI, 5.02–25.41) per 100,000 person-years. Considering a window period of 16 days, the estimated relative risk for repeat donors was 7.35 (95% CI, 4.16–27.66) window period units transfused per 1,000,000 donations, or one WP donation derived unit transfused per 136,054 donations. The estimated global incidence rate was 12.8 window period units transfused per 1,000,000 donations.
Summary/Conclusions: The residual risk of HIV in the studied population could be considered moderate-high, compared to lower values reported in Europe and the USA, and higher values reported in Africa and Brazil itself. This study reports the only and most current estimation of the residual risk of HIV in Colombian blood donors.

3B-S07-03
RESIDUAL RISK OF CYTOMEGALOVIRUS AFTER LEUKOREDUCTION IN THE CANADIAN BLOOD SUPPLY

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Background: Risk from leukocyte associated viruses such as Human T-cell Lymphotropic Virus (HTLV) and Human Cytomegalovirus (CMV) can be reduced by leukoreduction. Both HTLV and CMV cause lifelong infection, usually asymptomatic. Up to 10% of HTLV infected individuals may eventually develop clinical disease. All cellular products undergo pre-storage leukoreduction. All donations are tested for HTLV antibody. However, the safety benefit of HTLV testing of all donations is

unclear. Currently CMV antibody negative product is provided on request for high risk patients, but the cost effectiveness of this option has been questioned.
Aims: To estimate the residual risk of CMV and HTLV in leukoreduced cellular products. In the case of HTLV there are two scenarios: 1-testing all donations for antibody to HTLV, or 2- testing only first-time donations.
Methods: Key assumptions were that risk correlates with filter failure, and is due to incident cases (both HTLV incident and prevalent cases if not tested). The residual risk was the product of the probability of filter failure [p(f)] and probability of the unit containing infectious particles [p(viremia)]. P(f) was the number of filter failures (residual white blood cell count of 5 x 106 or greater) for a product divided by the number of units tested in 12 months. P(viremia) for HTLV was the observed incidence in repeat donations adjusted for first-time donations, and for the second scenario was the incidence multiplied by the mean number of years of donation (6 years) to include donors who seroconvert and continue to donate. P(viremia)for CMV was obtained from publications. Confidence intervals were estimated as the product of the 97.5% confidence limits for p(f) and p(viremia).
Results: HTLV P(viremia) was estimated for scenario 1: 0.54 per million donations; scenario 2: 1.4 per million first-time donations, 1.88 per 100,000 person years, repeat donors. CMV P(viremia) was estimated as 0.12%. There were 10 filter failures of 8,057 RBC's (0.001241157), 6 of 1,207 pooled platelet (0.004971002), 5 of 1,409 apheresis platelet (0.003548616). For HTLV scenario 1 the residual risk was 1 in 1.5 billion RBC's (95% CI: 1,311.6–21.9 billion), 1 in 909 million platelets (95% CI 16.2 million–18.7 billion). For scenario 2, 1 in 47.2 million RBC's (95% CI 7.4–696 million) and 1 in 2.9 million platelets (95% CI 0.38–59 million). The residual risk of CMV was 1 in 679,810 RBC's (95% CI: 1,979,022–280,347) and 1 in 185,667 platelets (95% CI: 762,777–62,814).
Summary/Conclusions: The residual risk of HTLV in leukoreduced products would be very low if only first time donations were tested. The residual risk of CMV in leukoreduced products is also very low (without antibody testing), but a few recipients may be exposed each year. About 40% of donors have positive antibody tests for CMV antibody, thus many recipients are likely also to have been already infected and are immune. Most recipients are at low risk of clinically significant sequelae from either infection. Thus the chance of receiving an infectious unit AND becoming infected AND developing clinically significant disease is very small.

3B-S07-04
DISCOVERY OF "FALSE HIV ELITE CONTROLLERS" AMONG SOUTH AFRICAN BLOOD DONORS

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Background: The South African National Blood Service (SANBS) excludes HIV positive donations by performing pre-donation questioning of all donors and screens all donations for HIV antibody and HIV RNA by nucleic acid testing (NAT) in parallel. This testing algorithm allows the identification of prevalent HIV infections (antibody+/NAT+), incident HIV infections (antibody-/NAT+) and potential HIV elite controllers (antibody+/NAT-). HIV elite controllers (EC) maintain HIV viral load at very low levels without any antiretroviral therapy, presumably due to inherent immune response. During counselling and recruitment of potential EC into a cohort study, there was anecdotal disclosure by potential EC of prior knowledge of HIV infection and antiretroviral therapy (ART) use at the time of donation.
Aims: The aim of this study was to understand the extent of this "false EC" phenomenon and generate hypotheses for its genesis and prevention.
Methods: Blood donations were tested for HIV antibody (PRISM, Abbott) and HIV RNA using individual donation NAT (Procleix, Grifols). Stored plasma of donations that tested as potential EC was tested for five ART drugs using qualitative liquid chromatography–tandem mass spectrometry (sensitivity 0.02 μg/ml). We compared the frequency of "false EC" against blood drive characteristics, small donor incentives and the temporal trend of ART rollout in South Africa using chi-square, Fisher exact and trend tests.
Results: From 2010-2015, SANBS identified 152 HIV antibody+/NAT- donors as potential EC and sent stored plasma samples from these donations for ART drug testing. Unexpectedly, 103 (67.8%) had detectable levels of ART drugs (88 Efavirenz, 9 Nevirapine, 6 Lopinavir, 0 Darunavir, 0 Atazanavir) and were therefore considered "false EC"; the remaining 49 (0.56% of all HIV infections identified) were considered true EC. There was no difference in the occurrence of false EC at fixed (46%) vs. mobile (58%) blood drives (P = 0.14) nor during periods in which small gifts were offered (75%) or not offered (63%) to encourage blood donation [P = 0.76]. There was a trend toward increasing frequency of false vs. true EC by year from 2010 through 2015 [P = 0.057], coinciding with the number of South Africans receiving ART.
Summary/Conclusions: False EC due to undisclosed ART use, represent a large and growing proportion of all potential EC identified in South Africa. The phenomenon does not seem to be associated with small blood donation incentives but may be increasing as ART coverage increases in South Africa. With the advent of ART initiation at diagnosis, the risk of seroreversion may be a threat to blood safety as HIV positive donors on treatment may test antibody- /NAT-. Finally, other countries with hyper endemic HIV burdens and good ART coverage may experience false EC with implications for HIV research and risk to blood safety.

3B-S07-05
THE NATIONAL BLOOD SERVICE AS A PLATFORM FOR HIV CURE RESEARCH: A PRELIMINARY REPORT FROM THE MONITORING AND ACUTE TREATMENT OF HIV STUDY (MATHS)

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Background: All blood donations in South Africa are tested in parallel for HIV antibody (Ab) and RNA using highly sensitive individual-donation nucleic acid testing (ID-NAT). About 60 South African donors per year are detected to have acute (less than 21 day duration) HIV infections (AHI) (RNA+/ Ab-; Fiebig stages I and II). A larger number of recent HIV infections (RNA+/Ab+; Fiebig stages III to VI) (RHI) of less than 5–6 months duration are also identified. We hypothesized that the rapidity of anti-retroviral therapy (ART) initiation would correlate with smaller HIV reservoirs and increase eligibility for future "HIV Cure" interventional research.
Aims: To assess the impact of ART initiation in AHI and RHI on HIV disease progression including virologic and immunologic response and to evaluating the successful linkage to care of blood donors identified with early HIV infection.
Methods: A prospective cohort study will enrol 50 AHI and 25 RHI donors detected at the time of blood donation. HIV Ab (Abbott Prism HIV O Plus) and HIV RNA ID-NAT (Grifols, Emeryville, CA) are measured on samples taken at donation and again at enrolment. RHI is detected by a limiting antigen (LAg) avidity assay (Sedia, Portland, OR). In collaboration with the Clinical HIV Research Unit, ART with Raltegravir/Tenofovir/Emcitrabine is initiated at enrolment and switched to Efavirenz/Tenofovir/ Emcitrabine at 6 months. Leukocytes from peripheral blood and leukapheresis are tested for HIV genotype, ART resistance and HIV reservoir (HIV proviral DNA and cell-associated RNA).
Results: From October 2015 to February 2017 we enrolled 29 donors with AHI (25 evaluable for demographics and clinical laboratory results, 27 evaluable for Fiebig staging), median age 25 years, 16 females, majority Black, median HIV RNA 285,707 copies and mean CD4 459 cells/mm^3. Enrolment occurred a median of 12 days after donation and ART was initiated a median of 2 days after enrolment. Fiebig staging at donation was 16 and 11 respectively for stages I and II and at enrolment it was 3, 10 for stages I and II; and 14 for stages III to VI. Viral suppression (<20 copies/ml) occurred after a median of 35 days on ART. The 11 (7 evaluable) enrolled RHI cases had similar demographics, lower median viral load (5,101 copies) and slightly higher CD4 counts (489 cells/mm^3) than the acute group. Viral suppression was achieved at a median of 28.5 days. Two cases of transmitted Efavirenz resistance were managed by continuation of the Raltegravir regimen.
Summary/Conclusions: This study provides proof of principle that a partnership between a national blood service and a treatment NGO can be used to detect and rapidly treat persons with AHI and RHI in South Africa. Initial results suggest that around 47% of AHI enrolees were still in Fiebig stage I/II at enrolment and that rapid viral suppression can be achieved once they are started on ART. Similar HIV research may be undertaken in other countries with active HIV epidemics and blood services using parallel screening for HIV antibody and nucleic acid.

3B-S07-06

INCIDENCE OF HTLV-1 AND HTLV-2 IN UNITED STATES BLOOD DONORS, 2008–2016

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Background: Human T-lymphotropic virus types 1 and 2 (HTLV-1 and -2) are human retroviruses that cause adult T-cell lymphoma and myelopathy. We have previously reported HTLV-1 prevalence of 5.1 per 100,000 persons and HTLV-2 prevalence of 14.7 per 100,000 in first-time US blood donors from 2000–2009 Chang et al. J Infect Dis 2014). Although prevalence studies can indicate areas of endemicity and risk groups for HTLV-1 and -2 infection, only incidence can highlight foci of ongoing transmission. The latter is important for monitoring the current epidemiology HTLV infection as well as success of prevention strategies.
Aims: To measure HTLV-1 and-2 incidence among U.S. blood donors.
Methods: We used data from a large blood collection network in the western and southern United States covering the period 2008–2016 when a similar confirmatory algorithm was in use. HTLV-1 and -2 antibody screening was performed with a HTLV-1 and-2 antibody chemiluminescent assay (Abbott Diagnostics, Lake Forest, IL) and repeat reactive samples were confirmed with the INNO-LIA HTLV I/II Score assay (Innogenetics, Ghent, Belgium). Blood donors with at least two donations during this period contributed data, with person-years calculated as the time between the first and last donations (negatives) and time between the first negative donation and the midpoint between the last negative and incident positive donations (positives). Incidence was calculated as the number of confirmed positive donations divided by the sum of negative and positive person years.
Results: Among 1,095,061 donors followed for 2,917,021 person-years, there were 16 incident HTLV infections including 6 HTLV-1, 3 HTLV-2 and 7 HTLV untypeable infections. Overall incidence using the INNO-LIA algorithm was 5.5 (95% CI 3.1 – 8.9) per million person-years (MPY); it increased to 8.6 (95% CI 5.6–12.6) per MPY if samples positive under a dual EIA algorithm were counted. Incidence was 4.0 per MPY in males and 7.0 per MPY in females and highest in the younger age groups (10.1 per MPY in donors aged 15–24, 5.3 per MPY in those aged 25–44 and 3.1 per MPY in those aged 45 and older). Donors of non-White race (9.8 per MPY) had higher incidence than those of White race (4.4 per MPY).
Summary/Conclusions: Among U.S. blood donors, HTLV-1/2 incidence of 5.5 per MPY is lower than the 15.9 per MPY reported by the REDS network in 2000 but higher than the 2.1 per MPY reported among American Red Cross donors in 2012. It is substantially lower than HTLV-1 blood donor incidences reported in Japan (Satake Lancet Infect Dis 2016) and Brazil (Carneiro-Proietti AIDS Res Hum Retrov 2012), probably due to the higher burden of HTLV infection in those countries. Differences in HTLV-1/2 incidence by sex and racial group are consistent with HTLV epidemiology and higher incidence in young people is worrisome as it suggests ongoing transmission by sexual contact or injection drug use. We conclude that blood donor screening for HTLV is a useful platform for measuring incidence of this human retrovirus.

Donors/Donation: Donor Psychology

3B-S08-01

THE EMOTIONAL PSYCHOLOGY OF BLOOD DONORS: UNDERSTANDING AND USING THE AFFECTIVE KEY TO DONOR RETURN

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Introduction: Donor retention is a key concern for blood collection agencies around the world. Traditional approaches to understanding why donors do not return have used rational models of cognitive decision-making, such as the Theory of Planned Behaviour. Yet this research has yielded few successful interventions. We suggest that insufficient attention has been paid to donors' emotional experiences while donating, with research failing to consider the breadth of positive and negative emotions that donors may experience during the different phases of the donation process. Identifying and understanding these emotions may be key to developing effective strategies to boost donor retention. As such, our research addresses this gap by measuring the emotional experience of blood donors before, during, and after their donation, and through tracking the impact of that experience on return behaviour.
Methods: First-time and novice whole blood and plasma donors (N = 945) were recruited while they attended a donation appointment. Donors reported in real-time their current levels of joy, pride, fear, stress, and sadness at four time points: in the waiting area of the donation centre, in the phlebotomy chair before and after needle insertion, and in the refreshment area after donation. In the refreshment area, donors also reported their satisfaction with the waiting time before donation as well as their intention to return to donate. Donor behaviour was tracked over the following 6 months.
Results: Preliminary results indicate that positive emotions were far more prevalent than negative emotions. In all donor groups, the highest levels of fear and stress were reported in the phlebotomy chair before needle insertion, whereas the highest levels of joy and pride were reported in the refreshment area. Satisfaction with the waiting time correlated positively with joy experienced after donation across the entire sample. Among first-time whole blood donors, positive (but not negative) emotional experience predicted intention, with initial analyses suggesting that positive states in the waiting area impacted likelihood of return. Among novice whole blood donors, both positive and negative emotional experience arising at several time points predicted intention and the experience of these emotions in the waiting area predicted return. Novice plasma donors' emotional experience predicted neither intention nor return. For first time plasma donors, however, both positive and negative emotional experience influenced intention, but only fear in the phlebotomy chair after needle insertion undermines return.
Conclusion: Our research provides unique insight into the dynamic nature of donors' emotional experiences, documenting their experiences of both positive and negative states as they arise before, during, and after donation. Findings from this initial study will allow the design and evaluation of innovative interventions (e.g., drawing on the Process Model of Emotion Regulation) to amplify those emotional states identified as promoting return while dampening emotional states that deter return. As such, this research not only augments our understanding of the donor's in situ experience, but will also lead to theory- and evidence-based retention strategies.

3B-S08-02

PERSONAL CHARACTERISTICS OF BLOOD DONORS INFLUENCE THEIR VOLUNTARY NON-MEDICAL WITHDRAWAL

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Background: Each year, approximately 10% of the Dutch blood donors become inactive. Inactivation is recorded in the donor registry for varying reasons. The most common reasons are medical (e.g. repeated low Hemoglobin levels), reaching the upper age limit for donating, inactivation on donor's own request, and non-response to repeated invitations. Social cognitions and personality have been linked to a wide variety of illness behaviours including voluntary non-compliance.
Aims: Hence, we investigated whether social cognitions and personality characteristics, measured before the first donation, would similarly predict donor voluntary non-medical withdrawal.
Methods: In the Netherlands, donors must register and attend a comprehensive medical eligibility interview prior to their first donation. Using the Dutch donor registry, we randomly assigned new, eligible donors to receive a blood donation survey in the period July 2008–March 2009. Questionnaires were sent ten days prior to recipients' first appointment at the blood bank. A total of 4861 new donors received a questionnaire accompanied by a letter explaining the study aim and emphasizing that the questionnaire should be returned before their medical interview. Two thirds of recipients completed the questionnaire (N = 2964; ~61%). Besides demographic background, measures included personality characteristics, blood donation intention, norms and attitudes, and donation related anxiety. Furthermore, we measured expected planning failure, anxiety and need for information about donation process and blood use. The inactivation codes retrieved from the Dutch donor registry comprised inactivation on donor's own request for personal reasons, non-response to repeated invitations, donor cannot be reached, and donor no-show after invitation.

To assess associations between donor characteristics and withdrawal from the donor pool by the end of 2016, we fitted univariate and multivariate Cox regression models, including confounding factors, such as age, gender and blood group.
Results: Out of 2964 donors who filled in the questionnaire before their first donation in 2008 or 2009, more than one third (36.5%) was inactivated due to non-medical reasons by 2016. The other donors either continued their donor career (47.5%) or stopped donating due to medical reasons (16%). Univariate logistic regression models showed that withdrawal negatively associated with positive norms (OR = .91, P < .01), attitudes (OR = .78, P < .001) and intentions (OR = .80, P < .001) towards blood donation, higher self-efficacy (OR = .80, P < .001) and more life time donations (OR = .76, P < .001). Voluntary non-medical withdrawal was further associated with higher anxiety (OR = 1.13, P < .001) and expected planning failure (OR = 1.22, P < .001) prior to the first donation. Multivariate Cox regression models, taking into account the different times donors had been active until the occurrence of either withdrawal or continuing to donate by the end of the study, revealed that withdrawal was driven by anxiety (OR = 1.05, P = .06) and need for information (OR = .93, P < .001).
Summary/Conclusions: Specific individual characteristics including social cognitions and personality traits of donors increase their risk of voluntary withdrawal from the donor pool for non-medical reasons. Especially donors with higher donation anxiety and more need for information about the procedure and blood use had higher risks of voluntary inactivation. These donors might benefit from extra attention and information provision during their first donation.

3B-S08-03

FACTORS AFFECTING RETURN OF FIRST-TIME DONORS AFTER A SUCCESSFUL, UNEVENTFUL BLOOD DONATION

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Background: Retention of first-time donors (FTDs) is important to maintain the adequacy of the blood supply. Deferrals and negative experiences are known factors that negatively impact donor return.
Aims: To identify factors associated with FTD return after a successful, uneventful donation.
Methods: Individuals successfully donating to a large US blood collector for the first time in 2014 were followed for 732 days (24 months). The analysis was restricted to those without deferral who completed a donation unassociated with an observed or reported adverse reaction. We report pertinent descriptive statistics and present a multivariable logistic regression analysis to identify factors associated with return at least once in the subsequent 24 months. Donor characteristics (sex, age, race, ABO/Rh type, BMI, EBV) and donation factors (phlebotomy, sponsor, and site types) were included in the regression model.
Results: Of 141,435 FTDs, 99,860 were not deferred and completed a donation without a reaction; 53% of the latter were male, 82% donated whole blood and 16% 2-RBCs, the balance, various multicomponent apheresis procedures; 40% were 16–18 and 45% were 23–64 years old; 50% were Group O. The overall return rate was 49%. The mean number of presentations in 24 months by all returning donors was 2.3 (SD 1.9, median 2, IQR 2); apheresis platelet donors presented 5.2 times (SD 6.9, med. 3, IQR 5). Donors at fixed sites presented 3.3 times (SD 3.3, med. 2, IQR 3) and donors 65+years old 3.0 times (SD 2.6, med. 2, IQR 3). Donors more likely to return [odds ratio, 95% CI] were: female [1.09,1.05–1.14], 16-17 and 65+ compared to 23–64 year olds [2.2, 2.1–2.3 and 1.4, 1.3–1.5, respectively], O-negative compared to A-positive [1.2, 1.2–1.3], obese and extremely obese compared to overweight donors [1.09, 1.05–1.13 and 1.1, 1.02-1.19, respectively], donating at college and at the workplace compared to community sponsor groups [1.09, 1.04–1.15 and 1.07, 1.02–1.11, respectively], and donating at fixed sites compared to mobile set-up sites [1.53, 1.46–1.61]. Donors less likely to return were: 18 and 19–22 compared to 23–64 year old donors [0.79, 0.75–0.84 and 0.74, 0.7–0.8, respectively], AB-positive and AB-negative compared to A-positive [0.9, 0.8–0.95 and 0.8, 0.7–0.98, respectively], low- and high-normal BMI compared to overweight donors [0.87,0.84–0.91 and 0.94, 0.9–0.97, respectively], Black non-Hispanic and Hispanic compared to White non-Hispanic donors [0.73, 0.69–0.77 and 0.84, 0.82–0.87 respectively], and donating in a mobile bus compared to mobile set-up sites [0.84, 0.81–0.86].
Summary/Conclusions: Identification of factors associated with higher or lower return rates in donors after a successful donation can guide the development of recruitment strategies. In the absence of national data systems, and in a mobile population, this information should be interpreted in the proper context, as a donor who does not return to one blood collector could have returned to another.

3B-S08-04

A LIFE COURSE PERSPECTIVE ON BLOOD DONATION: THE INFLUENCE OF LIFE EVENTS ACROSS THE DONOR CAREER

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Background: All over Europe, blood donor numbers are decreasing. Although this does not pose a short-term threat, since the demand is decreasing too, demographic developments (e.g., ageing and growing diversity within the population) may negatively affect the blood supply on the long-term. Hence, recruitment and retention of blood donors remain an important topic of study. For several decades, researchers have been studying donor behaviour while trying to characterize the 'typical blood donor'. However, findings on determinants of donor behaviour are inconclusive, with results changing over time and varying within and between countries.
Aims: We aimed at getting a more thorough understanding of the dynamic nature of blood donor behaviour by adapting a 'life course perspective'. This approach shifts the focus from the static *current state* of blood donor behaviour to dynamic *donor careers*: 'How does blood donor behaviour and motivations change during the life course, and how do life events impact these behavioural and motivational change?' Previous studies using retrospective, cross-sectional designs indicated that life events might serve as turning points in donor careers. Self-reported reasons for donors to lapse or reduce their frequency of donation include childbirth, time constraints due to work or study, moving and living farther away from a donation centre, and changing job status.
Methods: For statistical analyses, two data sources were used: 1) Dutch Donor Database (eProgesa), containing information on all donors and their behaviour (e.g., donation frequency, return rates), and 2) Donor InSight (DIS I: 2007–2009; DIS II: 2012–2013), a large-scale survey, including information on donor characteristics, motivations, and life events. The survey data was linked to Donor Database records to describe relationships between life events (occurred between DIS I and DIS II), and blood donor lapse (retrieved from eProgesa at the time of DIS II).
Results: A total of 22,132 donors participated in both DIS I and DIS II. Of these donors, 22.5% (n = 4,983) had stopped donating at the time of DIS II. Preliminary analyses show that donors who, in the period between DIS I and DIS II, were widowed, had a higher chance to stop donating compared to donors who were not widowed in the same time-frame (Exp(B) = 2.18, 95% CI = (1.63, 2.78), P < .000). Donors who married showed higher stopping risks compared to donors who remained unmarried (Exp(B) = 1.29, 95% CI = (1.11, 1.51), P = .001). Divorcing had no influence on donors' stopping risk. Regarding labour market related events, results suggest that donors who lose their job or retire have a higher stopping risk than donors who stayed in their job (respectively Exp(B) = 1.69, 95% CI = (1.42, 2.01), P < .000; Exp(B) = 3.08, 95% CI = (2.84, 3.35), P < .000). Donors who found a job had lower stopping risks than donors who remained unemployed (Exp (B) = 0.65, 95% CI = (0.50, 0.85), P = .001).
Summary/Conclusions: Life events do seem to have an influence on blood donor behaviour. Widowhood, marriage, job loss, and retirement are all associated with a higher stopping risk, while getting a job reduces the risk of stopping. Further analyses will show if, and how, these (and other) life events impact donor frequency and donor motivations, and whether effect vary between donors form different socio-demographic groups.

3B-S08-05

FACILITATORS AND BARRIERS FOR RHD-IMMUNIZED WOMEN TO BECOME AND REMAIN ANTI-D DONOR

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Background: Since the introduction of routine postnatal (1969) and antenatal prophylaxis (1998) with anti-RhD Ig in the Netherlands, combined with additional anti-RhD Ig in high-risk situations during pregnancy and delivery, the number of women, newly immunized by pregnancy and delivery, has declined significantly. In the Netherlands, the majority of current anti-D donors consists of these naturally immunized women. Hence, the success of the prevention program has resulted in a decreasing availability of new anti-D donors. Influx of new donors, however, is necessary to maintain a sufficient pool of anti-D donors.

Aims: In this study, we investigated motivators, barriers, predictors and appreciated recruitment strategies for anti-D donorship in RhD immunized women, who are potentially eligible to become anti-D donor.
Methods: A mixed-methods design was applied, including qualitative focus groups discussions and quantitative questionnaires. The focus group discussions (two including 11 women) served as input for the development of the questionnaire. Both the focus groups and questionnaires included current anti-D donors and potential anti-D donors. The topics identified in the focus groups and included in the questionnaire were: demographic characteristics, prosocial behavior (for example, registered as an organ donor) and values (such as altruism), pregnancy and hemolytic disease of fetus and newborn (HDFN) and attitude toward anti-D donorship.
Results: We invited 750 anti-D donors and potential donors to complete the questionnaire. The overall response rate was 47.6%; 50.4% were anti-D donor, 38% potential donors and 11.6% ex-donors. Almost 70% of the non-donors would become a donor if they knew about the possibility, half of them wanted more information first. Reported disadvantages were time investment and travel time investment; half of the donors mentioned no disadvantages. Motivators of non-donors for anti-D donorship were "want to do something back" (31.2%) and "want to prevent having a sick child or losing a child for others" (33.9%). Multivariable analysis revealed that single women (OR 5.8 $P = 0.02$) and women with partner without resident children (OR 7.9 $P = 0.03$) were more likely to be an anti-D donor compared to women living together with a partner and children. Not being registered as organ donor (OR 0.25 $P < 0.001$) or leaving the choice to relatives (OR 0.41 $P = 0.05$) was a significant predictor for not being an anti-D donor. Also the experience of severe HDFN was a significant predictor, but this might be, at least partly, explained by selection bias, due to the recruitment of non-donors from the LUMC.
Summary/Conclusions: The main barrier for women with RhD antibodies to be an anti-D donor was the lack of knowledge about anti-D donorship. Positive predictors of anti-D donorship were living without resident children and altruism. Negative predictors were not being registered as organ donor and possibly the severity of the experienced HDFN. The blood bank should develop targeted recruitment strategies with the focus on spreading knowledge about anti-D donorship among RhD-immunized women.

Cellular Therapies-CAR T cells

3B-S09-01
NEW TECHNOLOGIES AND APPLICATIONS FOR CAR T CELL THERAPY

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Adoptive immunotherapy with T cells that were modified by gene-transfer to express a tumor-targeting chimeric antigen receptor (CAR) is being investigated as a novel and transformative way for treating cancer. CARs are synthetic receptors with an extracellular antigen-binding domain derived from the VH/VL chains of an antibody, an intracellular signaling domain – most commonly CD3zeta *in cis* with a co-stimulatory domain such as CD28 or 4-1BB, and recognize surface molecules independent from HLA. The CAR-transgene can be inserted into autologous or allogeneic T cells to provide a personalized tumor-reactive T-cell product for an individual patient.
Clinical trials at centers in the US have demonstrated the curative potential of this approach with dramatic and durable complete anti-tumor responses in a subset of patients with chemo-radiotherapy refractory $CD19^+$ B-cell acute and chronic leukemia (ALL/CLL) and non-Hodgkin lymphoma that received T cells modified with a CAR specific for the B-lineage marker CD19. Importantly, there are several factors that affect efficacy and safety of this treatment including the specific design of the CD19-CAR, the composition of the CAR T-cell product with subsets of killer and helper T cells, and the lymphodepleting conditioning regimen that is administered prior to infusion. Additional strategies to improve the currently achieved outcomes include the use of CD19-CARs with humanized targeting domains to reduce immune-mediated rejection of CAR T cells, and combination therapies with checkpoint inhibitors.
An ongoing effort in the field is to identify and validate alternative tumor antigens to extend applications of CAR T-cell therapy. Several CAR target antigens are in advanced pre-clinical and/or early clinical development including CD20, CD22 and CD37 in lymphoid malignancies, CD33 and CD123 in myeloid malignancies, and BCMA, CD38 and SLAMF7 in multiple myeloma. Our own group is further pursuing the ROR1 molecule that is not only expressed in CLL and mantle cell lymphoma but also epithelial cancers, including triple-negative breast cancer and lung adenocarcinoma. We have demonstrated the ability of ROR1-specific CAR T cells to confer anti-tumor reactivity in pre-clinical models.
There is a strong clinical need and desire the increase the availability of CAR T-cell therapy at centers in Europe. We have recently developed an augmented gene-transfer strategy based on *Sleeping Beauty* transposition that eliminates the need for viral gene-transfer vectors and has the additional benefit of a safer genomic integration profile to minimize the risk for malignant transformation and genotoxicity. We are in the process of establishing the GMP manufacturing process for CAR T cells using this new gene-transfer strategy and are preparing clinical trials to implement this powerful new therapeutic modality at our institution.

3B-S09-02
ADOPTIVE CELL THERAPY BASED ON TUMOUR INFILTRATING LYMPHOCYTES

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During the last decade the field of cancer therapy has been reformed by the development of new and effective treatment modalities with a major focus on immune therapeutic strategies. Beside the successful immune check point antibodies different forms of adoptive cellular immunotherapies have been exploited in the past years. Among these adoptive cell therapy (ACT) based on the infusion of ex vivo expanded tumor infiltrating lymphocytes (TILs) has been the most applied and successful.
TIL based ACT is a personalized treatment defined as the infusion of T cells harvested from autologous fresh autologous tumor tissues after ex vivo activation and extensive expansion. The final cell infusion product consist of a polyclonal T cell population comprising a highly variable amount and diversity of tumor specific T-cells. The expanded TILs are infused i.v. after 1 week of intensive lymphodepleting chemotherapy and followed by treatment with high i.v. doses of IL-2.
TIL based ACT has yet only been tested in smaller phase I/II studies but these studies consistently confirm an impressive clinical response rate of up to 50% in metastatic melanoma including a significant proportion of patients with durable complete tumor eradication - response rates able to compete with the immune check point antibodies.
Other approaches might contribute to the improved efficacy of TIL therapy. These strategies could specifically aim at increasing the anti-tumor activity of T cell products, or at improving host conditioning for a better in vivo TIL survival and tumor targeting by immune sensitization of tumors. Furthermore, initiatives to extend TIL therapy to other diagnoses are ongoing.

3B-S09-03
THE THERAPEUTIC FUNCTIONS AND MECHANISMS OF EX VIVO INDUCED AND EXPANDED POLYCLONAL HUMAN STABLE CD8+ REGULATORY T CELLS ON AN AUTOIMMUNE DISEASES MICE MODEL: COLLAGEN-INDUCED ARTHRITIS MICE

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Background: Autoimmune disease (AID) such as rheumatoid arthritis (RA) is an immune system disease which is painful and can cause serious destruction to the human body. Research shows that regulatory T cells (Tregs) in AID patients are deficient or dysfunctional and transfusing Treg has an efficient therapeutic function on AID models. However, the mechanisms of Tregs cell therapy on AID patients are not revealed exactly. Additionally, due to the limited number of $CD4^+$ Treg and its unstable characteristic in inflammation (harmful to cell therapy), it is needed to amplify the stable Treg in vitro to investigate the therapeutic functions and mechanisms.
Aims: We proposed a method that could induce and expand sufficient stable $CD8^+$ Tregs and aim to investigate the therapeutic mechanisms of these Tregs on an AID mice model (CIA mice).
Methods: Collagen-induced arthritis (CIA) mice were induced with type-two collagen. Human $CD8^+$ T lymphocytes (from PBMC) were cultured with anti-CD3/28 beads and IL-2, induced with TGF-β1 + rapamycin in vitro and were re-stimulated

for another 3 rounds to get adequate Tregs. The stability of $CD8^+$ Tregs when encounter with inflammation in vitro were tested by Foxp3 expression, Th1 and Th17 cells conversion in inflammations (IL2 + TGF-β1 + IL21 + IL23 and IL2 + TGF-β1 + IL1β+IL6). $CD8^+$ Tregs survival and their Fopx3 expression after transfusion were investigated for the $CD8^+$ Tregs stability in vivo. $CD8^+$ Tregs were transfused into CIA mice and the therapeutic functions were evaluated. Additionally, the therapeutic mechanisms of $CD8^+$ Treg on CIA mice were tested by: 1. The subtype of mice $CD4^+$ T cells (mCD4) in mice spleen and the mRNA expression in mice foot after Tregs treatment; 2. Cell co-cultured assay in vitro: $CD8^+$ Tregs were co-cultured with $mCD4^+$ T cells or mice DCs (mDCs), the $CD8^+$ Tregs suppression on $mCD4^+$ T cells proliferation, the down-regulation of mDCs CD80/86 expression and the influence on $mCD4^+$ T cells differentiation were investigated.

Results: Ex vivo induced and expanded human $CD8^+$ Treg were $Foxp3^+IL17A^-$, which were stable in inflammations, expanded 10000 times and adopted vigorous suppression ability in vitro assay. $CD8^+$ Tregs therapy could significantly alleviate the severity of diseases (clinical score, level of anti collagen IgG antibody and cartilage destruction in CIA). In vivo, $CD8^+$ Treg treatment significantly reduced $CD4^+IL17A^+$ T cells and increased mice self $CD4^+Foxp3^+$ Tregs in CIA spleen and decreased IL17A, RANK1 and MMP-1 mRNA expression in CIA foot. In vitro co-cultured assay, we found $CD8^+$ Treg can efficiently inhibit $mCD4^+$ effect T cells proliferation and induce their apoptosis, increase % of $Foxp3^+$ Tregs and decrease % of $IL17A^+$ T cells in $mCD4^+$ T cells subtype and reduce CD80/86 expression on mDCs, revealing the fact that through these ways the severity of disease can be alleviated. Moreover, the $CD8^+$ Tregs were found in CIA mice foot (27.4 ± 2.03%), blood (4.55 ± 1.03%) and spleen cells (1.90 ± 0.05%) 72 h after transfusion and their % of $Foxp3^+$ were remained.

Summary/Conclusions: The results revealed that ex vivo induced and expanded human $CD8^+$ Tregs have an effective therapeutic function on AID models and are stable in inflammations and transfusion. Moreover, we also revealed the mechanisms of this therapeutic function. This research can provide some instructive reference and produce a novel stable cell for potential cell therapy on AIDs and also improves the utilization of blood component.

3B-S09-04

ROLE OF PLATELET RICH PLASMA INJECTION GRAFTS IN OSTEOARTHRITIS: A RANDOMIZED CONTROLLED TRIAL

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Background: Platelet rich plasma and mesenchymal stem cells are known to have a potential for articular cartilage regeneration. Specific growth factors have been proposed as therapeutic proteins for cartilage repair. Hypothesis: Platelet-rich plasma (PRP) provides symptomatic relief in early osteoarthritis (OA) of the knee. Study Design: Prospective, Randomized controlled trial; Level of evidence, 1.

Aims:

1. The purpose of this study was to assess the safety and efficacy of intra-articular injection of autologous Platelet rich plasma for knee osteoarthritis.
2. Clinical assessment of effect of PRP in osteoarthritis patients after one, two and three intra-articular injections.

Methods: A total of 108 patients (216 knees) with bilateral OA were divided randomly into 4 groups. Group A includes 116 knees received a single intra-articular injection of PRP, group B includes 66 knees received 2 injections of PRP with the interval of 4 weeks, group C includes 24 knees received 3 intra-articular injections of PRP with the interval of 4 weeks and group D includes 10 knees received a single injection of normal saline. White blood cell (WBC)–filtered PRP with a platelet count 5 times that of baseline was administered in all. All the groups were homogeneous and comparable in baseline characteristics. Clinical outcome was evaluated using the Western Ontario and McMaster Universities Arthritis Index (WOMAC) questionnaire before treatment and at 6 weeks, 3 months, and 6 months after treatment. They were also evaluated for pain by a visual analog scale, and overall satisfaction with the procedure.

Results: Statistically significant improvement in all WOMAC parameters was noted in groups A, B and C within 6 week, 3 month, 6 month and lasting until the final follow-up at 1 year. The mean WOMAC scores (pain, stiffness, physical function, and total score) for group A at baseline were 10.02, 3.01, 37.26, and 50.16, respectively, and at final follow-up were 6.49, 2.28, 21.68, and 28.17, respectively, showing significant improvement. Similar improvement was noted in group B (mean WOMAC scores at baseline: 10.46, 3.27, 38.26, and 51.18, respectively; mean WOMAC scores at final followup: 5.69, 1.97, 24.48, and 31.33, respectively). In group C, maximum improvement noted (mean WOMAC scores at baseline: 10.12, 3.40, 38.10, and 52.80, respectively; mean WOMAC scores at final followup: 5.02, 1.28, 18.12, and 25.17, respectively). In group D, the mean WOMAC scores deteriorated from baseline (10.24, 2.68, 32.86, and 49.54, respectively) to final follow-up (11.27, 3.68, 41.28, and 56.24, respectively). The 4 groups were compared with each other, and no improvement was noted in group D as compared with groups A, B, C (P\.001). There was significant difference in terms of improvement amongst groups A, B and C.

Summary/Conclusions: A single dose of WBC-filtered PRP in concentrations of >5 times the normal amount is as effective in early knee OA. The group C is given 3 doses of PRP was the most beneficiary amongst all studied group. The results, however, deteriorate after 1 year. All three groups treated with PRP had better results than did the group injected with saline only. It is strongly recommended that one intra-articular injection of PRP should be given annually after three intra-articular injections with the interval of 4 weeks.

Management & Organisation: Benchmarking

3B-S10-01

BENCHMARKING: PRINCIPLES AND PITFALLS

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Background: Measuring and managing performance is important to anyone - individuals, firms, and organizations. No matter how good we think we are, we can always be better. It requires, however, that we measure performance appropriately and understand what drives performance. In this way, we can learn better practices, make better decisions, and motivate improved performance.

Aims: Firms and organizations use multiple means to pursue multiple objectives. Moreover, the means and objectives interact in complicated ways. Two resources, say labor and capital, may for example both substitute and complement each other. Such interactions imply that simple, key performance indicators, including simple financial ratios, do not suffice to measure performance or guide decision making. Modern benchmarking therefore focuses on *comprehensive evaluations*, taking into account the simultaneous use of multiple resources to produce multiple services

Methods: We use recent advances in benchmarking, most notably the Data Envelopment Analysis (DEA) and Stochastic Frontier Analysis (SFA) methods, to support such evaluations. We introduce the underlying concepts and ideas without the usual mathematical wrapping. We demonstrate how to use state-of-the-art software, most notably *Interactive Benchmarking IB*, to support the evaluations and as a basis for managerial decision making.

Results: In this paper, we will investigate the benchmarking problem and what we consider to be the superior benchmarking tools. We will discuss the merits and, in particular, the problems in traditional benchmarking based on key performance metrics that oversimplify an organization, and we will show how state-of-the-art benchmarking moves far beyond the use of such simple metrics.

3B-S10-02

BENCHMARKING IN BLOOD TRANSFUSION: WHERE ARE WE NOW?

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Benchmarking, or comparison of practice between blood services has been gathering momentum over the last 15 years. The use of benchmarking, especially where blood services are able to identify their data sets, encourages the sharing of best practice and drives overall blood sector performance improvement in a largely non-competitive environment. Comparison of practice is now a feature of all major blood operator networks, including the European Blood Alliance (EBA), Alliance of Blood Operators (ABO), Asia Pacific Blood Network (APBN), and the Red Cross Global Advisory Panel (GAP) which includes some services in low and medium income countries (LMICs). A broad range of benchmarking approaches are adopted tailored to network needs, including the sharing of product and service costs, comparison of operational key performance indicators, corporate governance and risk management, and exchange of donor recruitment strategies. There are two primary objectives of

international benchmarking in blood transfusion: 1. To provide scientists, clinicians and managers with information about how to engage in available blood sector benchmarking programs, with a view to informing tactical and strategic decision-making. 2. To broaden the benchmarking agenda from operational performance comparison, to the generation of performance improvement opportunities. The benefits of international benchmarking have been significant for many of the participants. For example in Europe, the EBA Benchmarking Group (BMG) commenced its activities in 2006 and now comprises 12 core member organisations, with another 10 contributing their data to an annual scorecard process. The EBA BMG used the first 2 years of its life to ensure comparability of data definitions and fields, before commencing the process of data collection. The very wide range of data outputs was evident from an early stage. For example:- On session donor deferral rates varied from 3% to 25%- Collection productivity varied from 600 to 2,500 whole blood donations collected per annum per FTE (full-time equivalent) employed in blood collection activities- Red cells issued per thousand population served varied from 25 to 60A number of EBA's member services have identified improvement opportunities based on the benchmarking information. For example:- NHS Blood and Transplant (the blood service covering England), has used the "top quartile" collection, processing and productivity data to drive its supply chain performance improvement agenda. - The Danish Blood Services recognised that they were an outlier in respect of the quantity of red cells issued, and made strenuous efforts to reduce blood utilisation through a systematic approach to patient blood management. - The opportunities to improve testing productivity were recognised by a number of EBA members, who individually embarked on a series of automation and consolidation strategies designed to reduce costs. It is important to note that there are many opportunities for performance improvement that remain open to EBA members. The appetite to take advantage of such opportunities will inevitably vary, depending on the pressures imposed by funding agencies and other stakeholders in each jurisdiction.

3B-S10-03

OPTIMIZING STAFF ASSIGNMENT AT BLOOD COLLECTION SITES TO MINIMIZE WAITING

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Background: For every two to three whole blood donations, one working hour of a staff member at a blood collection site is required. This staff time does not include all additional activities, such as the medical check for new donors or efforts to recruit donors. As it is a labor intensive process, staff members should be used efficiently.

The staff time is required for three phases of the blood collection process: registration, medical screening, and blood donation. In the Netherlands, staff members are usually trained to work at any of these phases. All of these phases have a randomly distributed service time, which makes the optimal assignment of staff to the three phases non-trivial. Determining this assignment is made even more difficult by the random arrivals of blood donors. Whole blood donors do not have appointments for donations, and show clear preferences for particular times of the day, creating a random time-dependent arrival process.

Aims: We aimed to develop an algorithm to determine the optimal assignment of staff members to the three phases, to minimize queue lengths. This optimal assignment depends on the time of day and the number of donors present at the different phases of the collection site.

Methods: A mathematical model, a Markov Decision Process (MDP), has been developed that models a blood collection site. The MDP models both the stochastic behavior of services and arrivals, and the arrival patterns. Based on the current state of the process, the MDP minimizes the average expected queue length by computing the optimal assignments of the staff members to the phases at fixed time-intervals (e.g., every 60 or 450 seconds). To verify the results, a simulation model has been build that evaluates the implications of the optimal policy in a more realistic setting.

Results: The potential reductions of average queue lengths was assessed by numerical experiments with the MDP. Based on Dutch blood collection sites, these numerical experiments show that reductions of up to 80% on the average number of waiting donors throughout the day seem attainable. The simulation study shows reductions of up to 70% on the average number of waiting donors. The simulation model is also able to check waiting times, and also shows reductions of up to 70% on average waiting time.

Summary/Conclusions: With our model, it is possible to reduce the waiting time at a blood collection site by only reassigning staff members. Even without adding extra staff members, the average waiting time at a blood collection site can be reduced up to 70%.

3B-S10-04

PILOT EQA SCHEME FOR ROUTINE RED CELL GENOTYPING IDENTIFIES ERRORS IN TESTING AND REPORTING

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Background: The UK NEQAS (BTLP) red cell genotyping (RCG) pilot scheme was launched in 2016, following a pre-pilot project in collaboration with ISBT in 2014/15, where one of the outcomes was recognition that EQA for 'routine' red cell genotyping would be useful. Three exercises were distributed between June and December 2016, with 45 laboratories in 22 countries participating.

Aims: To assess the level of proficiency in testing and reporting genotypes and predicted phenotypes for common alleles in a routine patient testing context.

Methods: Each exercise comprised 2 whole blood EQA samples prepared from UK blood donations, selected only by Rh and Fy phenotype and presented as samples from haemoglobinopathy patients requiring genotyping to facilitate transfusion support. Laboratories reported results for D, Cc, Ee, MN, Ss, Kk, Fy^aFy^b, Fy, Jk^aJk^b and Do^aDo^b. Genotype and predicted phenotype responses were presented as tickbox selections using ISBT terminology, with an 'other' option, for use only if the result obtained could not be expressed using the ISBT options. There was an option to record results that would have been reported to clinicians using different terminology. Results were assessed vs. the consensus result for each allele. Error rates have been calculated based on 10 opportunities for error for genotype and 9 for predicted phenotype per sample. Additional questions regarding situations where genotyping is undertaken were included in June 2016.

Results: All 45 centres test patient samples for transfusion purposes. 2/6 samples distributed had a variant D allele, but in each case it was still possible to use a consensus result. 2629 genotyping results and 2376 predicted phenotypes were analysed. 29 incorrect genotypes and 24 incorrect predicted phenotypes were reported giving error rates of 1.1% and 1.0% respectively. 13 laboratories returned results outwith consensus, with 2 of these making errors in 2 exercises, and one apparently transposing 2 samples at some stage during testing or reporting. Excluding this transposition error, 18 sets of genotype/predicted phenotype results were outwith consensus: 6 for genotype only, 2 for predicted phenotype only, 9 for both and one where the predicted phenotype did not match the expected or reported genotype. Whilst the majority of results were returned using ISBT terminology, there were instances where alternative terminology was used, and where phenotypes were reported as genotypes and *vice versa*.

Summary/Conclusions: The pattern of results suggests that errors are potentially being made in testing, in interpretation of genotype to predicted phenotype and in reporting of results. Whilst the process for reporting EQA results is not the same as that for clinical results, transcriptions and interpretations are still made and it is likely that some of these errors could have occurred in clinical practice. The value of the Scheme would be enhanced by contacting participants to determine causes of error. It is important that the terminology is consistent between centres to avoid confusion in communication of results. We conclude that EQA highlights potential problems in clinical practice, and even 'routine' samples provide opportunities for educational points to be made.

Transfusion Practitioner Forum The Transfusion Practitioner effect on PBM adoption and implementation

TPS-01-01

CHALLENGES OF IMPLEMENTING A SUSTAINABLE PBM PROGRAM AND THE TRANSFUSION PRACTITIONER ROLE IN THE PROGRAM

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An important driver for PBM has been the high blood utilization in many western countries. Therefore, research and institutional PBM implementation programs have often targeted red cell transfusion, and how to control it properly. The TP role has evolved from the safety officer function (safe blood administration procedures), to an educating role, teaching transfusion guidelines, and supervising good clinical transfusion practices. Recently, red cell utilization has been reduced in many countries, and in parallel, there has been an increasing awareness of PBM. As a natural consequence, TPs have taken interest expanding their role to embrace PBM. This poses some fundamental questions: What is the spectrum of PBM, how can a sustainable PBM program be built, and how could the TP role be expanded to embrace PBM?

In Copenhagen we implemented a PBM program, from pilot project in one intensive care unit, to hospital and finally to the regional multicenter level. The main driver for this program was the liberal transfusion practice in the non-bleeding patients, and the high utilization. The program successfully addressed both doctors and nurses in clinical departments with significant transfusion activity. The intervention focused on education in transfusion guidelines and continuous data feedback on transfusion practices. PBM nurses (TPs) were a central element, and they were well received in the clinical departments. This approach proved a sustainable effect, which lasted for at least 3 years. After improving transfusion practice and reducing red cell usage, the program went on to improve perioperative bleeding control, and establish preoperative anaemia management. In spite of excellent results in treatment quality and a significant financial gain for the hospitals, the program was closed in 2016.

Throughout the lifetime of this PBM program, funding and stakeholder management have been substantial challenges. As PBM is for patients who may need transfusion, PBM stretches across many specialties and comprises patients from cradle to grave. TPs have an important role, as they represent a direct link to the clinical staff involved in diagnosis and treatment of both anaemic and bleeding patients. However, the TPs cannot stand alone in this vast landscape, but must be co-organized with doctors and multidisciplinary hospital teams involved in PBM. The PBM leading group must prioritize actions, specialties and patient groups, to sustain relevance, funding and effect. Strategic leadership and alignment across disciplines and institutions is crucial to sustain PBM, because the different stakeholders (hospital managers, quality and safety officers, clinical departments and blood banks) have different perceptions about the risks, benefits and economy of PBM.

TPS-01-02

IATROGENIC ANAEMIA, HOW THE TRANSFUSION PRACTITIONER CAN INFLUENCE

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Background: Iatrogenic anaemia is a condition in which a low haemoglobin concentration and haematocrit result from large or frequent venepuncture to patients for laboratory tests. It is also known as Hospital Acquired Anaemia.

The Patient Blood Management programme within the UK identified the minimisation of blood sampling from patients to reduce the risk of iatrogenic anaemia as a key objective. It has also been identified by the American Association of Blood banks (AABB) in their Choosing Wisely campaign and the Australian Patient Blood Management guidelines.

In the 2013 within the UK a Patient Blood Management Survey to hospitals demonstrated that only 21% of Hospital Trusts had a policy to minimise the volume and frequency of blood sampling to minimise iatrogenic anaemia. This number rose to 40% in the 2015 survey.

Aims: Transfusion Practitioners (TP) are key to driving the patient blood management (PBM) message within their clinical environments, and although some key areas within PBM may feel beyond the scope of control or influence of the TP, undertaking an iatrogenic anaemia project can deliver some positive changes that benefit patients, with an added benefit of possible financial gains due to reduction in both blood sampling and reduction in blood usage.

Methods: This talk will review how the TP can undertake an audit and collect data to understand the number of tests taken and the impact on patient's haemoglobin, how to engage with stakeholders in highlighting the benefits of this quality improvement project, consider what other work has previously been done on iatrogenic anaemia and discuss some potential strategies to reduce the volume of blood removed.

Results: By demonstrating the positive impact this can have, it adds momentum to the overall PBM programme within the hospital setting.

Summary/Conclusions: The TP role is not just about making big changes that alter clinical transfusion practice, but can be about making small positive incremental gains that contribute to the overall PBM picture.

TPS-01-03

THE TRANSFUSION PRACTITIONER ROLE IN MAINTAINING A SUSTAINABLE O NEGATIVE SUPPLY

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O negative red blood cells (RBC) are universal and used as emergency blood supply or first line treatment in urgent bleeding situations. Internationally overall demand for RBC is reducing, however, in many countries the demand for O negative RBC remains constant or has increased. Demand is often much greater than the proportional donor pool. In Australia the general O negative donor pool is 9% whereas demand is currently at 16%, in the United Kingdom (UK) and Canada the donor pool is between 6–7% with demand at 12%.

As O negative RBC demand is increasing there are concerns about sustainability of supply, and as such a variety of interventions/best practice recommendations have been engaged in many countries. The UK 'Save 1 O D Neg a week' promotion. The promotional toolkit includes a variety of resources such as a flyer, banner, infographic PowerPoint slides, good practice guidance documents and top 10 tips. The Canadian Blood Service contacted top users of O negative RBC and asked about their best practices of its use and compiled a position paper 'Utilization and inventory management of group O Rh(D)-negative red cells'. Best practice tips include developing and implementing a policy for haemorrhaging patients whose blood group is not known and policies for optimal inventory management.

In Australia, the National Blood Authority's 'Managing Blood and Blood Product Inventory: guidelines for Australian health providers' helps guide practices to reduce unnecessary wastage of blood and blood products. In parts of Australia, models have, or are being developed to review the allocation and management of emergency O negative RBC supply, especially supplies in rural/remote areas. In these areas it is a challenge to balance risk versus need where there are variations in clinical requirement and great distances between the health service and supporting laboratories.

The transfusion practitioners (TP) plays a key role in supporting these interventions/best practice recommendations as they are often the key interface between the clinical areas and the laboratory.

Activities that TP readily undertake include: coordination and input to the development and subsequent education of massive transfusion/critical bleeding protocols (MTP), review and audit of MTP activations to identify gaps, report findings, and undertake required actions. Involvement in the development and ensuing education of policy using O positive RBC in males and females not of child-bearing age or potential. Audit and report the use of O negative RBC outside MTP activation, and the number of O negative units transfused to non O negative patients. Audit of emergency O negative RBC storage, handling and stock rotation, report findings and undertake activities to ensure appropriate stewardship. TPs actively share tools and resources, and have a key role in promoting and sharing success stories.

The skills of the TP to communicate and effect change are extremely important to engage stakeholders to continue activities to support appropriate use and stewardship to sustain the scarce O negative RBC supply.

Immunobiology of blood cells: The HLA system

3C-S11-01

THE CLINICAL RELEVANCE OF THE HLA SYSTEM IN BLOOD TRANSFUSION

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Background: The major histocompatibility complex (MHC) is a region of the human genome containing many genes which have immune functions. The human leucocyte antigens (HLA) genes, found within the MHC, are the most polymorphic genes in humans and this polymorphism is related to the role of HLA gene products in immune responses to pathogens. Immune responses to HLA incompatibility between recipients and donors is an important factor in some of the serious hazards of transfusion such as Transfusion Related Acute Lung Injury (TRALI), Non Haemolytic Febrile Transfusion reactions, Transfusion Associated Graft vs Host Disease and Immunological Platelet Refractoriness.
Aims: The provision of HLA selected blood products for transfusion can help avoid adverse transfusion reactions but in TA-GvHD sharing of HLA haplotypes is an important risk factor. New approaches to HLA matching may improve outcomes in patient transfused with HLA selected blood products.
Methods: Advances in laboratory methods for HLA antibody definition such as Luminex single antigen technology has enabled patients with complex antibody profiles to receive HLA selected transfusions without the need for lengthy laboratory investigations with cell panels. In addition it is well documented that high resolution (HR) HLA matching in haemopoietic stem cell transplantation matching has been facilitated by the application of Next Generation Sequencing (NGS) for HLA typing. NGS technology can now been to applied donor typing for the provision of HLA selected platelets using HLA epitope matching (HEM).
Results: HEM algorithms are more effective when donors are HR HLA typed as there is no requirement to use frequency based analysis to impute the HR type based on low resolution typing information. This epitope rather than an antigen based approach to matching patients and donors may be more biologically relevant.
Summary/Conclusions: The increasing awareness of the clinical relevance of HLA in blood transfusion is highlighted by the emerging use of HLA selected red cell transfusion as part of the management of renal patients awaiting live transplantation. Although the higher resolution of the new HLA testing techniques may appear to add another level of complexity, they do allow improved definition of HLA types and epitopes which facilitates the selection of HLA compatible blood products.

3C-S11-02

PREDICTED INDIRECTLY RECOGNIZABLE HLA EPITOPES (PIRCHE) AND HLA-SPECIFIC IMMUNE RESPONSES

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HLA-specific antibodies can be detected in patients who have been exposed to allogeneic HLA after transplantation, blood transfusion or pregnancy. The presence of these donor-specific HLA antibodies before transplantation or transfusion is strongly associated with an impaired outcome. The ability of maternal B cells to produce HLA-specific antibodies highly depends on help from CD4+ T cells. B cells initially acquire and process the mismatched donor HLA and present the processed HLA epitopes to T helper cells via HLA class II molecules. Subsequently, antigen-specific T cell recognition drives proliferation and differentiation of naive B cells into memory B cells and plasma cells and facilitates IgM-to-IgG isotype switching. We have constructed a computational model on these HLA class-II epitopes to estimate the likelihood of donor-specific antigen development. This model calculates the Predicted Indirectly ReCognizable HLA Epitopes (PIRCHE) of mismatched HLA. Various studies in organ transplantation and pregnancy have shown that the number of PIRCHE associates with donor-specific antigen development. Given the high variability of the HLA system and the limited donor population, finding a fully HLA-matched donor for each recipient is virtually impossible. These recent developments of epitope-based matching principles for T-cell epitopes, possibly in combination with B-cell epitope models, may provide options to reduce the risk for donor-specific antibody development and to broaden the number of immunologically matched donors.

3C-S11-03

EFFECTIVE TREATMENT OF MURINE TRANSFUSION RELATED ACUTE LUNG INJURY (TRALI) USING INTERLEUKIN (IL)-10

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Background: Transfusion-related acute lung injury (TRALI) is the leading cause of transfusion related fatalities and is characterized by acute respiratory distress following blood transfusion. A two-hit model is generally assumed to underlie TRALI-pathogenesis. The first hit is a predisposing patient factor such as inflammation while the second hit can be conveyed by anti-leukocyte antibodies which are present in the transfused blood. Unfortunately, there are no specific treatments available for TRALI. Using novel murine TRALI models, we have shown that the first hit can be conveyed by a loss of an inhibitory response elicited by CD4 + T regulatory cells or dendritic cells, while the second hit can be delivered by anti-major histocompatibility complex (MHC) class I antibodies. These TRALI-reactions could be prevented by prophylactic administration of the anti-inflammatory cytokine interleukin (IL)-10 (Kapur et al. Blood 2017, DOI https://doi.org/10.1182/blood-2016-12-758185).
Aims: To test if IL-10 administration can rescue an ongoing TRALI reaction and if so, to determine what mechanism IL-10 elicits the protection.
Methods: We utilized a C57BL/6 murine TRALI model by first depleting CD4 + T cells followed by injection of TRALI-inducing anti-MHC class I antibodies (34-1-2s + AF6-88.5.5.3). After 15 minutes when TRALI symptoms occurred (defined as at least a 2 degree drop in rectal temperature), we then injected either IL-10 (45 μg/kg iv) or volume-matched PBS and subsequently assessed lung damage by measurement of pulmonary edema (lung wet-to-dry weight ratios; W/D). In addition, we measured several physiological TRALI associated parameters such as plasma levels of the neutrophil chemoattractant macrophage inflammatory protein (MIP)-2, pulmonary neutrophil accumulation and bone marrow or pulmonary neutrophil reactive oxygen species (ROS) production.
Results: Control mice injected with PBS exhibited a high degree of pulmonary edema as assessed by significantly elevated lung wet-to-dry weight ratios (W/D: 5.84 ± 1.02). Plasma MIP-2 levels and pulmonary neutrophil levels were also found to be increased. Bone marrow and pulmonary neutrophils were capable of potent ROS production and lung tissue histology confirmed severe signs of acute lung injury. In contrast, mice injected with IL-10 completely recovered from TRALI: they displayed no pulmonary edema (W/D: 4.76 ± 0.04, $*P < 0.02$ compared to mice injected with PBS) and no signs of severe acute lung injury assessed by lung histology. Plasma MIP-2 levels and pulmonary neutrophil levels, however, were equally increased in both groups. Surprisingly, IL-10 treated mice were also equally capable of potent ROS production by bone marrow and pulmonary neutrophils. Preliminary data suggests that IL-10 may be acting directly on the pulmonary endothelium.
Summary/Conclusions: IL-10 significantly rescued an ongoing TRALI reaction in mice without affecting the levels of plasma MIP-2, pulmonary neutrophil levels and neutrophil ROS production. IL-10 may be exerting its TRALI-protective effects towards the pulmonary endothelium and may prove to be a novel effective and feasible therapeutic strategy in combating TRALI.

3C-S11-04

THE DANISH BLOOD DONOR STUDY IS A SUITABLE COHORT FOR THE EXAMINATION OF ALLERGY AND ASTHMA RISK FACTORS

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Background: Allergic rhinitis and asthma are common chronic diseases with substantial impact on quality of life. Studies using the European Community Respiratory Health Survey (ECRHS) questionnaire found prevalences of 24% for allergic rhinitis and 10% for asthma in adults.

The development of allergy and asthma depends on environmental factors; e.g., exposure to microbial compounds in early life (upbringing on farm with livestock), which lowers the prevalence of atopy. The susceptibility of an individual is nevertheless modified by the genetic background, and genetic variations of great importance for antigen presentation can be found in the Human Leukocyte Antigen (HLA) region. In The Danish Blood Donor Study (DBDS) it is possible to explore associations of allergy/asthma to both set of risk factors.

Aims: The aim was to assess whether the DBDS population is a suitable cohort for the study of airborne allergy and asthma.

Methods: DBDS is a prospective population-based cohort study and bio bank. From May 2015 until May 2016, 13,400 blood donors completed a questionnaire, including standardized questions about allergic rhinitis, asthma and upbringing, based on the ECRHS questionnaire. For a total of 1,485 participants, genomic HLA data were available through the Danish Bone Marrow Donor Register. Data were analyzed using adjusted logistic regression models.

Point prevalences of allergic rhinitis and asthma were determined; and associations between allergy/asthma and previous risk factors, including place of upbringing and HLA class II, were analyzed.

Results: The cohort included 1,129 participants (8.48% (8.00-8.95)) with asthma, of whom 95% reported that the diagnosis had been confirmed by a doctor; and 2,572 participants (19.36% (18.69-20.03)) with allergic rhinitis. Livestock farm upbringing was associated with lower prevalence of allergic rhinitis than city upbringing (OR=0.65 (0.54-0.79)), as well as for the combination of nasal and eye allergy (OR=0.53 (0.42-0.67)). An urban−rural gradient across the urbanization levels was observed (especially among women). The risk of asthma was non-significant for livestock farm upbringing compared to city upbringing (OR=0.89 (0.68-1.16)).

For women, the individual HLA alleles DQB1*03:02; 06:02 and DRB1*04:01; 11:04; 15:01 were positively associated with asthma (OR=1.88, 1.95, 1.94, 4.87 and 1.84 respectively, $P < 0.030$ for all), whereas protective effects were found for HLA-DRB1*03:01 (OR=0.42, $P = 0.022$). For both sexes, HLA-DRB1*11:01 was positively associated with allergic rhinitis (OR=2.39 (women) and 1.95 (men), $P < 0.016$).

Summary / Conclusions: Compared to other studies based on ECRHS questions, the prevalence of allergic rhinitis and asthma was lower; but the proportion of confirmed asthmatics was similar. Our results support a protective effect of livestock farm upbringing compared to city upbringing, with respect to the risk of allergic rhinitis. Furthermore, we were able to demonstrate positive as well as negative associations with specific HLA class II antigens.

On this basis, we conclude that the DBDS blood donor population is suitable for the study of allergy and asthma.

Blood products: Alternative treatments

3C-S12-01

PLATELETS IN LIVER REGENERATION

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Background: Loss of functional liver tissue leading to acute liver failure represents a major cause of morbidity and mortality. Currently, whole liver transplantation constitutes the unique treatment for patients not recovering with standard supportive care. Understanding the mechanism of liver regeneration and developing therapies to sustain liver regeneration are of high therapeutic relevance. In this regard, platelets, which play crucial roles in inflammation and wound healing and contain biologically active molecules that can potentially affect hepatocyte proliferation, were considered as potential candidates for stimulating liver regeneration.

Aims: We aim to review the most recent evidences regarding the role of platelets in liver regeneration.

Methods: Review of the literature.

Results: Platelets stimulate liver regeneration and improve survival in animal models of liver resection. In humans, platelets are independent predictors of post-operative mortality, liver function and volume recovery. The first proposed mechanism by which platelets stimulate liver regeneration relies on a direct effect of platelets on hepatocytes. Following partial hepatectomy, platelets are recruited to the liver and release their content. Platelet-contained molecules, such as HGF, VEGF, IGF-1 and serotonin, stimulate hepatocyte proliferation. An alternative mechanism involves the transfer of platelet mRNA to hepatocytes following platelet internalization, leading to hepatocyte proliferation. The second potential mode of action of platelets relies on their interactions with liver sinusoidal endothelial cells, which constitute a major regulatory element for hepatocyte proliferation. Platelets induce the secretion of IL-6 from liver sinusoidal endothelial cells, which is a strong initiator of hepatocyte proliferation. Also, platelets convey extracellular nucleotides and molecules with angiogenic properties, that may impact liver sinusoidal endothelial cell regulation and, by extension, liver regeneration.

Summary / Conclusions: Platelets stimulate liver regeneration. Several mechanisms seem to be involved, at the levels of hepatocytes and liver sinusoidal endothelial cells. These effects are mediated by the platelet content. Identification of the molecule(s) involved may lead to therapies for patients with impaired liver regeneration or needing improvement of the regenerative response.

3C-S12-02

IDENTIFICATION OF PROCOAGULANT PLATELET SUBSETS IN COLD-STORED AND CRYOPRESERVED PLATELETS USING A NOVEL CELL DEATH MARKER

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Background: Conventional platelet concentrate storage is at room temperature (22–24°C) with a shelf life of only five days. Cold-storage (2–6°C) and cryopreservation (−80°C with DMSO) would allow extension of the shelf life and ease supply pressures, and reduce expiry of unused stock. However, current literature suggests these methods lead to platelet activation and microparticle release, which raises concerns of increased risk of thrombosis following transfusion of these products. Furthermore, the effect of storage on the development of procoagulant platelets – implicated in pathological thrombosis – remains unclear. A novel cell death marker, GSAO, can be used to identify procoagulant platelets (Hua, Blood 2015) and assess the effects of platelet storage.

Aims: To evaluate the impact of different modes of storage on the activation and procoagulant profile of platelet concentrates.

Methods: Three ABO/RhD-matched, irradiated buffy coat-derived platelets were pooled and re-split into three matching concentrates. Each was subjected to conventional storage (RT), cold-storage or cryopreservation (n = 6 in each arm). Testing occurred on day 1 (baseline), days 2, 5, 9 and 14 for RT and cold-stored platelets and immediately post-thaw (after reconstitution) for cryopreserved platelets. Testing included platelet count, mean platelet volume (MPV; CELL-DYN Emerald, Abbott), thrombin generation (Thrombinoscope BV) and measurement of the proportion of $CD62P^+$ (activated) and $CD62P^+/GSAO^+$ (procoagulant) platelets in resting and in collagen, thrombin or dual-stimulated platelets by flow cytometry (BD FACSCanto II). Data were analysed using paired *t*-tests, where $P < 0.05$ was considered significant.

Results: Both cold-stored and cryopreserved platelets had significantly higher MPV than RT at all time points ($P < 0.05$), although platelet counts were not significantly different. CD62P expression was significantly increased in resting cold-stored and cryopreserved platelets (mean day 5 $CD62P^+$: RT 41%; cold-stored 79%; post-thaw 63%; $P < 0.001$), although this difference was lost after thrombin +/− collagen stimulation. Interestingly, cryopreserved platelets could not be further activated by agonists and were significantly less activated than maximally stimulated RT and cold-stored platelets at all time-points ($P < 0.001$) Cryopreservation led to a marked increase in procoagulant platelets compared to RT and cold-stored platelets at all time points, both at rest and following thrombin +/− collagen stimulation (resting $CD62P^+/GSAO^+$: baseline 0.3%; cryopreserved 25.6%; $P < 0.001$). In resting and stimulated cold-stored platelets, there was a significant increase in the procoagulant sub-population on days 9 and 14 compared with RT storage, whereas platelets under both storage modes had similar proportions at earlier time points. Cryopreserved platelets reached peak thrombin generation significantly faster than both RT and cold-stored platelets at all time points, and more thrombin was generated in cryopreserved and older cold-stored platelets. Endogenous thrombin potential was unchanged between storage modes.

Summary / Conclusions: Cold-storage and cryopreservation of platelet concentrates have a significant impact on the activation and procoagulant profile of platelets, whereby cold-storage is a potent activator and cryopreservation induces a large proportion of procoagulant platelets. The clinical impact of these storage effects remains to be elucidated.

3C-S12-03

EFFECT OF PATHOGEN INACTIVATION ON MICRO-RNA PROFILE OF PLATELET CONCENTRATES DURING STORAGE UNDER STANDARD BLOOD BANKING CONDITION

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Background: The Platelet has a central role in hemostasis and represents an integral part of transfusion medicine. Platelets can be stored in gas permeable plastic containers under agitation for a maximum of 5–7 days. Two main reasons for this limited storage time are a risk of pathogen contamination and an onset of platelet storage lesions (PSL). PSL is a collective term of variety of factors that contribute to the deterioration of platelet quality during storage. To reduce the risk of pathogen contamination, methods have been developed that render pathogens inactive in platelet concentrates prior to storage. The most widely used method is the INTERCEPT™ pathogen inactivation method, which is based on cross-linking nucleic acids using amotosalen and UV light.
MicroRNAs (miRNA) are small none coding 19-24 nucleotide long RNA molecules that serve as post transcriptional regulators of gene functions by binding to mRNA′s and facilitating translation inhibition or degradation of mRNA. Several publications support the notion of miRNA being important in platelet function. Changes in the regulation of specific miRNAs during storage have been reported as well as perturbation effects related to pathogen inactivation methods.
Aims: To investigate the effects of Intercept treatment on selected miRNAs in buffy coat generated platelets stored for 7 days under standard blood banking conditions.
Methods: Platelet concentrates were produced using the double dose (DD) buffy coat (BC) method. Using a pool and split strategy 4 identical single dose units where generated that originated from 24 whole blood donors. Each sister unit received different treatment (Intercept/Control-SSP+/Irradiated in plasma/Control-Plasma). Platelets units where sampled on day 1, 2, 4 and 7. *In vitro* quality was monitored using 20 different quality control (QC) parameters. A selection of 30 miRNA based on previous miRNA array study and the literature on their roles in platelet biology and storage was analysed with QPCR.
Results: A notable difference was found in several QC parameters relating to treatment but overall the four groups where compatible in their performance in QC analysis. Out of the 30 miRNA tested only three showed a significant difference at one or more time points as a result of different treatment. On day 7 miR1260a and miR1260b were downregulated in groups Control-SSP+, Control-Plasma and Irradiated, Control-Plasma respectively when compared to day 1 baseline samples. miR-96-5p was downregulated on day 2 and 4 in the Intercept group compared to Control-SSP+.
Summary / Conclusions: The INTERCEPT treatment does not change the quality or significantly alters the miRNA profile of platelet concentrates generated and stored using standard blood banking condition.

3C-S12-04

QUALITY CONTROL OF COLD-STORED APHERESIS PLATELET CONCENTRATES

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Background: In connection with an ongoing clinical trial of cold-stored platelets for the treatment of bleeding in patients undergoing cardiothoracic surgery at Haukeland University Hospital in Norway, in vitro quality controls were performed to ensure that platelet concentrates meet the EU requirements of >40 ml per 60×10^9 platelets, minimum 200×10^9 platelets per unit, $<1 \times 10^6$ leukocytes per unit and pH >6.4 measured at 22°C.
Aims: To evaluate in vitro quality and platelet function of leukoreduced apheresis platelet concentrates in platelet additive solution stored at 2–6°C for 14 days.
Methods: 32 platelet concentrates were collected using the Trima Accel Automated Blood Collection System *(Terumo BCT)* with PAS-III *(Intersol, Fenwal)* (n = 4) or PAS-III M *(T-PAS+, Terumo BCT)* (n = 28) as storage medium (65% PAS, 35% plasma). After a 2 h rest in room temperature, platelets were moved to storage at 2–6°C with agitation for a period of 14 days. Samples were taken on days 1, 7 and 14 to measure platelet count *(Cell-Dyn Sapphire, Abbott Diagnostics)*, residual white blood cell count *(BD Leucocount™ Kit, BD Biosciences)*, the metabolic measures pH (22°C), glucose and lactate *(ABL800 FLEX, Radiometer Medical)* and platelet function evaluated by thromboelastography *(TEG 5000, Haemonetics Corporation)* and arachidonic acid-induced impedance aggregometry *(Multiplate ASPItest, Roche Diagnostics)*. Bacterial growth (*BacT/ALERT FA, bioMérieux*) was measured on days 7 and 14. As previous publications and own experience show, the swirling phenomenon is not present in cold stored platelets. Therefore this visual test was not performed.
Results: 32 apheresis platelet concentrates were collected during the study period, of which one unit was excluded from analysis due to an apheresis procedure error. The mean volume on day 1 was 241 ± 8.5 ml with 45.4 ± 4.0 ml per $60x10^9$ platelets (n = 31). Platelet count decreased from 323 ± 30 $x10^9$/unit (n = 31) on day 1 to 317 ± 30 (n = 28) and 308 ± 32 (n = 28) on days 7 and 14. Residual white blood cell count was 0.16 ± $0.12x10^6$/unit (n = 21). pH remained stable during the first week between 7.28 ± 0.03 (n = 29) to 7.25 ± 0.07 (n = 26), with a slight decrease to 7.15 ± 0.10 (n = 27) after 2 weeks. Glucose decreased from 6.3 ± 0.6 (n = 29) to 4.7 ± 0.9 (n = 26) and 3.2 ± 1.1 (n = 27) mmol/l, while lactate increased from 2.1 ± 0.4 (n = 29) to 5.5 ± 0.9 (n = 26) and 8.2 ± 1.5 (n = 27) mmol/l. Thromboelastography showed stable values with R-time (8.3 ± 1.2 (n = 31) day 1, 8.7 ± 1.1 (n = 26) day 7, and 9.6 ± 1.3 (n = 24) day 14), angle (76.7 ± 5.0 day 1 (n = 31), 78.2 ± 4.0 (n = 25) day 7, and 76.1 ± 4.3 (n = 24) day 14), and maximum amplitude (MA) (65.3 ± 6.4 (n = 31) day 1, 67.7 ± 7.1 (n = 26) day 7, and 68.9 ± 6.8 (n = 24) day 14) for 14 days. Multiplate ASPItest was stable for seven days (AUC 86 ± 10 (n = 29) on day 1, 83 ± 14 (n = 28) on day 7) with a decline to 56 ± 18 (n = 28) on day 14. Bacterial tests were negative for all units on days 7 and 14.
Summary / Conclusions: Cold-stored platelet concentrates meet the quality requirements when stored agitated for up to 14 days. Changes in pH and metabolic parameters reflected the expected low metabolism. When evaluated by thromboelastography and impedance aggregometry, platelet function was preserved during storage for up to 14 days.

3C-S12-05

MEASUREMENT OF MECHANICAL PROPERTIES OF RED BLOOD CELLS POST-STORAGE USING OPTICAL TWEEZERS

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Background: The study of mechanical properties of red blood cells (RBCs) is of interest, given their high deformability in the circulation and changes in these properties during routine storage at 4°C. It is known that these properties are affected in storage by a reduced supply of ATP and slowing of the metabolism. Limited work has been done on the mechanical properties of RBCs when returned to a physiological environment post-storage. The principle of optical tweezers is that momentum carried by photons exerts a force opposite to their trajectory when hitting an object which can then be trapped and manipulated. This technique has been used to measure mechanical properties of cells, especially deformability and elasticity, through stretching.
Aims: To quantify elasticity evolution in the RBC membrane when placed back into plasma after storage and to utilise optical tweezers to measure the influence of morphology on deformability.
Methods: RBCs were sampled from four units of red cells at weekly intervals during routine storage and placed into ABO compatible human pooled plasma to model a physiological environment. A typical optical-tweezers arrangement was used, with a 1,070 nm wavelength laser light. The optical trap used a 60x water immersion, high numerical aperture (NA = 1.20) microscope objective. Stretching was realised by optically trapping a cell between two lasers and increasing the distance separating the beams by 0.13 μm every second until the cell escaped. The force exerted as well as images used to measure cell diameter were recorded. Discocytes and echinocytes found in plasma were investigated.
Results: The force required to stretch cells after 5 weeks of storage was on average 5.62 ± 3.49 pN/μm (3.12 ± 0.80 pN/μm for discocytes, 8.13 ± 3.34 pN/μm for echinocytes). The stretching of the RBC membrane did not follow a linear elastic deformation, but showed 'energy thresholds' where the force required to maintain a stretched diameter decreased suddenly by up to 2 pN. This energy threshold phenomenon was observed for 73.3% of echinocytes tested and 38.3% of discocytes.

Data are currently being collected for previous and following time points up to 50 days of storage.
Summary / Conclusions: This study discovered energy thresholds in the deformation of RBCs under a tensile loading. These observed 'energy thresholds' were attributed to a reorganisation of the cytoskeleton to reduce the stress on the membrane resulting in a rapid increase in cell diameter. The evolution in elastic behaviour during storage, linked to intracellular reorganisation in this study, provides new insights into this process. Analysis after reconstituting the cells in plasma is a model for the physiological change post-transfusion. Optical tweezers proved to be a powerful tool for the discovery of modifications related to cytoskeletal reorganisation.

3C-S12-06
APPLICATION OF FLOW CYTOMETRY TO EXAMINE CHANGES IN RED BLOOD CELLS DURING STORAGE

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Background: There have been numerous statistical clinical studies on the effects of the storage of red blood cells on its recipients and especially among patients with cardiac and neurological disorders. Transfusiologists most frequently have studied biochemical changes in erythrocytes stored in blood banks. In transfusion medicine flow cytometry is mainly used to evaluate feto-maternal hemorrhage, rarely to measure the determinants of group antigens or antibodies or to study microvesicles in relation to red blood cell aging.
Aims: Evaluation some surface molecules that are important in the activity and survival of young and stored red blood cells, their susceptibility to hemolysis by phagocytosis, changes of complement inhibitors and osmotic fragility.
Methods: Samples from blood units after 2, 28, and 42 days of storage (20–40 samples per group).
Methods: The flow cytometer FACSCanto II (BD) and antibodies: anti-CD44, CD47, CD55, CD58, CD59, CD235a (GPA – Glycophorin A) were used to study the expression of surface molecules, anti-CD14 and anti-GPA to examine erythrocyte phagocytosis by monocytes; human anti-D (Bio-Rad) and eluates with warm IgG autoantibodies to opsonize red blood cells. Flow cytometry was used to evaluate erythrocyte osmotic fragility: percentage of residual erythrocytes were measured sequentially in real time after spiking with deionized water.
Results: 1. A significantly more intense MFI (mean fluorescence intensity) of CD44, CD47, CD55 and CD58 was observed at day 42 of storage than at day 2; the differences were not significant at day 28 and were not present in the leukodepleted samples. A statistically significant MFI decrease of GPA was observed on day 42 of storage of the red blood cells with leukocytes. There were no changes in the CD59 expression. There were two subpopulations of red blood cells with different fluorescence intensity for CD47 expression in both the leukodepleted and non-leukodepleted units. Their proportions changed differently but statistically significantly during storage: the subpopulation with more intense fluorescence decreased in the leukodepleted units and increased in units with leukocytes. 2. The phagocytosis of red blood cells coated with antibodies was measured by the phagocytosis index and was statistically significantly higher in stored (4 weeks) than young red blood cells. 3. Although the MCV was higher at 42 day than at day 2, osmotic fragility evaluation showed no differences between young and stored red blood cells.
Summary / Conclusions: 1. The increased removal of stored red blood cells after transfusion compared to young red blood cells may be the result of increased susceptibility to phagocytosis, decrease in GPA expression and changes in CD47 but was not the result of reduced osmotic fragility. 2. Flow cytometry can be a useful tool for searching the features and markers of stored red blood cells in blood banks.

Donors/Donation: Donor adverse effects

3C-S13-01
DONOR FAINTING: RISKS FACTORS AND PREVENTION STRATEGIES

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Prevention of fainting reactions associated with whole blood donation is an important issue for donor safety and donor retention. Such syncopal-type reactions are the most common adverse reactions in whole blood donors with a reported, but probably underestimated, frequency of 2–3% in the general donor population, but reaching 10% in the youngest donors. A large majority of fainting reactions occur immediately or shortly after the blood donation and 10% occur after leaving the donation site.
Mechanisms underlying syncopal-type reactions in blood donors are thought to be different according to the stage in the donation process: a neurocardiogenic (vasovagal) mechanism may lead to a syncopal-type reaction during or immediately after donation, while orthostatic intolerance exacerbated by relative hypovolemia may lead to syncopal-type reactions more than 10 min after whole blood donation.
To assess the relative importance of fainting risk factors, a retrospective case–control study of severe immediate and delayed fainting was performed. French hemovigilance data collected from 2011 to 2013 reports 8410 immediate and 833 delayed faintings occurring after a total of 8 834 214 donations. In multivariate analysis, occurrence of immediate fainting was strongly associated with first-time donation (OR 4,34; 95% CI: 3,93–4,79, P < 0,0001) and the 18–24 age group (OR 2,24; 2,00–2,45, P < 0,0001) and of delayed fainting with women with a normal BMI (OR 7,31; 4,96–10,77, P < 0,0001), overweight BMI (OR 7,89; 4,84–12,87, P < 0,0001) or obese BMI (OR 3,72; 1,42–9,74, P < 0.0001), and in men with an underweight BMI (OR 6,39; 1,56–26,13, P < 0,0001). Overall, first-time donation by a young person appears particularly at risk of immediate fainting while a female donor is at risk of delayed fainting.
To identify and quantity means to reduce donor fainting risk, we performed a cluster randomized prospective trial (EVASION trial) assessing the impact of three different pre-donation hydration strategies: 500 ml of an isotonic drink vs 500 ml of water and an advice to drink (control arm) coupled or not with applied muscle tension to prevent immediate and/or delayed fainting. The main outcome was the cumulative incidence for each arm of presyncopal (feeling faint) and syncopal reactions requiring a Trendelenburg position on the donation site or any transient interruption of the daily activities (e.g. to sit down) outside the donation site in 4576 whole blood donors.
Overall, faintness occurred in 5.5% of whole blood donation. Compared to the control arm (i.e. advice to drink), simple drinking 500 ml of plain water or an isotonic significantly reduced the rate of faintness [OR = 0.74; 0.55–0.99, P = 0.041], independently of muscle tension. Analysis according to time course of donation showed that: (i) muscle tension significantly reduced faintness during the donation [OR = 0.64; 0.42–0.98, P = 0,041]; (ii) Isotonic drink significantly reduced delayed off-site faintness [OR = 0.62; 0.40–0.98, P = 0.040] and tiredness after donation [OR = 0.75; 0.59–0.94, P = 0.014].
Overall, drinking 500 ml of an isotonic solution or plain water and muscle tensing, at time of blood donation, are useful measures to prevent fainting reactions overall. Furthermore, drinking an isotonic solution can provide a novel approach to reduce the risk of off-site delayed fainting reactions as well as tiredness after whole blood donation.

3C-S13-02
THE IMPACT OF IMMEDIATE VASOVAGAL REACTION ON DONOR RETENTION

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Background: Vasovagal reaction (VVR) associated with dizziness, sweating and nausea with or without loss of consciousness (LOC) is the most commonly reported reaction in whole blood donors, occurring in 2.9% of donations in Australia in 2016. The Australian Red Cross Blood Service (Blood Service) collects over 1.3 million voluntary

non renumerated donations every year, 60% of which are whole blood. VVRs are unpleasant for donors, present a risk of donor injury, and affect Blood Services (BS) operational capacity. VVRs have a significant negative impact on donor retention, both through permanent or long-term temporary deferral, but more importantly, through donor-self-deferral. Current Blood Service procedures recommend donor deferral for donors who experience loss of consciousness. Given the increasing demand for blood products, we need to assess whether donors who return to donate after experiencing a vasovagal reaction are at increased risk of further adverse events.

Aims: The aim of this review is to examine the impact of VVR on whole blood donor retention and to determine whether donors who have experienced a VVR can subsequently donate successfully.

Methods: We conducted a descriptive analysis of reported immediate VVR in whole blood donors who attended between 01/01/12-31/12/2016(n = 97,868). We extracted historical data from the donor database (eProgesa) to quantify donor retention following VVR. Data was analyzed to determine the impact of VVR with and without loss of consciousness and donor self-deferral was compared to Blood Service initiated deferrals.

Results: 97,868 immediate vasovagal reactions were reported over 5 years, from 3,906,485 whole blood donations, giving an incidence of 2.5 percent. 90,233 reactions (92 percent of VVR) were not associated with LOC. 54 percent of these donors have not returned to donate. Eight percent of donors who experienced a VVR were medically deferred, and 46% self-deferred. Of the 45,123 donors that have returned to donate 24 percent went on to successfully donate more than five donations. This accounted for over 95,000 donations from donors who had previously experienced an immediate VVR.

Summary / Conclusions: Blood Services have a duty of care for all donors. Medical deferrals of donors following VVR may be appropriate for a small group of donors with complications of VVR. Our review demonstrates, however, that many donors who return following a VVR can become successful long term donors and make a significant contribution to the increasing demand for blood products. This suggests that automatic deferral following VVR should not be standard practice; instead eligibility for future donation should be individually assessed.

To meet ongoing demand, donor retention is vital. In addition to efforts to prevent VVR prevention in all donors, blood services may focus their future efforts on targeted education of donors who return following VVR, to improve their self-efficacy and encourage return.

3C-S13-03

SERIOUS ADVERSE EVENTS OF BLOOD DONATION REPORTED IN NHSBT 2010-2016– TRENDS, THEMES AND LEARNING POINTS

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Background: NHS Blood and Transplant(NHSBT) collect approximately 1.7million blood donations annually. Blood donation is generally safe; donor complications sometimes do occur. Serious adverse events of donation(SAEDs)are those which either result in donor hospitalisation, interventions or significant disability persisting for >1 year post donation or, rarely, death- these are categorised as per the ISBT standards introduced in 2008 and revised in December 2014. These are duly reported to Medicines and Healthcare Products Regulatory Agency and investigated in a timely manner to identify any improvements that could be made to prevent them from occurring.

Aims: An analysis of reported SAEDs in NHSBT from January 2010 to December 2016 was undertaken to identify any emerging trends in the event types and recognise any areas for improvement.

Methods: All SAED reports logged from January 2010 to December 2016 inclusive were collated and analysed.

Results: A total of 239 SAEDs were reported during this period. An increase is noted in the rate of SAEDs reported per 10,000 donations from 0.16 in 2010 to 0.24 per 10,000 donations in 2016. The majority (63%) of SAEDs reported occurred in females with 49% (120) of the total SAEDs involving donors aged 45–64 years. Hospital admission within 24 hours of donation was the most common SAED reported at 48%(115) followed by donors suffering a fracture within 24 hours of donation at 22%(53). Problems relating to needle insertion persisting for >1 year contributed to 19%(46) of SAEDs. Three donor deaths occurred within 7 days of donation; however none were directly linked to donation. There were no reports of haemolysis or air embolism due to component donation.

Overall 71%(82) of hospital admissions following donation were related to a vasovagal reactions (VVR)- 65%(53) of these were related to delayed VVR while 35%(29) were due to faints at session. Majority(68%) of the donors admitted to hospital within 24 hours of donation were female. Of the 53 donors who sustained fractures, all were following VVR and 43(81%) of these donors were female. Damage to donor's teeth was seen in 41%(22) followed by lower limb fractures in 25%(13). There were 46 reports of donors with problems lasting over 12 months related to needle insertion, no significant difference was noted between male and female donors. Donors who suffer a serious adverse event are generally withdrawn from future donations, rarely have donors continued to donate.

Summary/Conclusions: Serious adverse events of donation are rare but do occur. They adversely impact donor satisfaction and retention and are not always preventable. Although there has been an overall reduction in the total number of VVR reported annually, these along with nerve injuries continue to be the most frequent SAEDs. Reporting processes have improved over the years and donors have been made more aware of the need to report any problems following donation; this may have influenced the number of events reported in the 7 year period. Further research into interventions designed to prevent or reduce VVRs in blood donors is needed to enhance donor safety and ensure sustainability of blood supply.

3C-S13-04

DISTRIBUTION OF FERRITIN LEVELS IN DUTCH DONORS – ARE FERRITIN LEVELS ASSOCIATED WITH LOW HAEMOGLOBIN DEFERRAL AT THE SUBSEQUENT DONATION?

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Background: Whole blood donation is associated with a loss of iron. To prevent iron deficiency a minimum haemoglobin (Hb) level is set as eligibility criterion and is measured before each donation. However, ferritin might be a more suitable parameter as it is an indicator of iron stores. Donor deferral is undesirable for donors who invest time and for blood supply organisations, as deferral is associated with costs and loss of donors. If ferritin levels are associated with low-Hb deferral at the subsequent donation attempt, ferritin might be useful to tailor donation intervals. This in turn might decrease deferral rates.

Aims: To map the distribution of ferritin and Hb levels in categories of number of donations in the Donor InSight-III population and new donors. Furthermore we aim to examine if low ferritin levels are associated with low-Hb deferral at the next donation attempt.

Methods: Donor InSight (DIS) is a cohort study among the Dutch donor population. During DIS-III (2015-2016) Hb and ferritin levels were measured. Ferritin levels were also measured in a sample of new and first time donors (2017). Data on previous donations were retrieved from eProgesa, the blood bank information system. Per category of donation history (0, 1, 2–10, 11–20, 21–30, 31–40, 41–50, >50 whole blood donations) mean ± SD ferritin (ng/ml) and Hb levels were calculated separately for men, premenopausal (≤44 years) and postmenopausal women (≥45 years). Participants who attempted to donate at least once after the initial ferritin measurement were included in the sub analyses to test the association of ferritin with Hb-related deferral. Ferritin levels were categorised into high ≥60, medium ≥30 and <60 and low <30 ng/ml levels. To test associations with subsequent low-Hb deferral, logistic regression was performed adjusted for age and stratified for sex.

Results: A total of 5048 participants (2828 DIS-III and 2220 new and first time donors) were included in these analyses. Mean Hb levels were similar for donors with varying donation histories. Ferritin levels of new donors were 135 ± 96, 50 ± 34 and 73 ± 47 respectively for male, premenopausal and postmenopausal women. Mean ferritin levels decreased with the number of donations and were approximately 50% lower for donors with more than 50 donations, compared to those with 2–10 donations. A total of 1809 participants returned for a subsequent donation attempt before the end of 2016 and were included in the sub analyses. The lowest ferritin category of women had 2.67 (95% CI 1.18–6.03) times higher odds of low-Hb deferral compared to the highest ferritin group of women. The lowest ferritin category of men had 11.83 (95% CI 2.70–51.89) times higher odds of low-Hb deferral compared to the highest ferritin group of men. Only for men the odds of low-Hb deferral increases significantly with age, odds 1.05 (95% CI 1.01–1.09)

Summary / Conclusions: Hb levels are relatively stable, ferritin levels drop gradually with the number of donations. Low ferritin levels seem to be associated with

Hb-related deferral at a subsequent donation attempt. These preliminary results suggest that interventions based on ferritin levels might help to prevent low-Hb deferral in the future.

3C-S13-05

APPLYING NEWER PARAMETER RETICULOCYTE HEMOGLOBIN EQUIVALENT(RET-HE) TO ASSESS LATENT IRON DEFICIENCY IN APPARENTLY HEALTHY BLOOD DONORS

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Background: Latent iron deficiency (LID) is also called iron deficiency without anemia (normal hemoglobin). It is important to assess LID since individuals with LID are likely to develop iron-deficiency anemia in weeks or months following diagnoses of LID, if not treated. Several studies from India show high incidence of Iron Deficiency Anemia (IDA) amongst blood donors especially regular blood donors. It is important to detect it early in such blood donors to maintain donor pool and avoid donor attrition. Early detection is difficult by measuring classical markers like hemoglobin (Hb), MCV and MCH as they detect iron deficiency relatively late. Conventional biochemical markers such as serum iron, transferrin or ferritin, may detect earlier than the classical markers but are often disturbed during inflammation (acute phase reactant) or in severe chronic diseases. Soluble transferrin receptor (sTfR) is presently the gold standard for diagnosing LID. However, sTfR also has certain limitations like requirement of batch processing, not routinely performed at most laboratories and being an expensive test.
Newer electronic hematology cell-counter offers a research parameter known as Reticulocyte Hemoglobin Equivalent (Ret-He) which detects iron deficiency at early stages. Ret-He is direct estimate of recent functional availability of iron into erythron. This test does away with the limitations of sTfR since it can be run on individual samples, is easy and inexpensive.
Aims: To evaluate the ability of Ret-He to predict LID in blood donors in comparison to sTfR.
Methods: It was a prospective longitudinal study, done on 501 randomized blood donor samples from October 2015 to September 2016 in department of Transfusion Medicine at a tertiary care hospital in India. The prospective blood donors were administered medical history questionnaire and brief physical examination in accordance with national guidelines. Donors were provided the information brochure of the study and those who consented were included in the study. Pre-donation Hb was estimated by photometer (Compo Lab TS) in finger-prick sample and those with Hb ≥12.5 were included. Additional clotted and anti-coagulated blood samples were collected during blood donation according to institutional SOP. All hemograms were performed on Sysmex XE-2100 analyzer which included Ret-He. Reference test sTfR was measured in batch assays by ELISA (Biovendor). Ret He <28 pg and sTfR≥3 μg/ml were used to diagnose LID. Serum Iron, TIBC and Serum Ferritin were also measured simultaneously.
Results: Of 501 blood donors (486 males and 15 females), 148 (29.5%; 139 males and 9 females) were having LID. Ret-He correlated with sTfR in 135 donors, thus accounting to sensitivity of 92.7%, specificity of 97.16%, PPV of 93.1% and NPV of 96.3%. Serum Iron, TIBC and serum Ferritin had comparatively lower sensitivity of 87.16%, 79.7% and 77.7% respectively.
Summary/Conclusions: Ret-He can be used as a reliable test for diagnosing LID among blood donors and its routine testing especially in regular donors could provide an opportunity to make appropriate interventions like dietary changes or drug supplementation and thereby avoid donor attrition.

Clinical: Clinical Transfusion 2

3C-S14-01

SHOCK INDUCED ENDOTHELIOPATHY (SHINE) IN ACUTE CRITICAL ILLNESS – A UNIFYING PATHOPHYSIOLOGIC MECHANISM

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One quarter of patients suffering from acute critical illness such as severe trauma, sepsis, myocardial infarction (MI) or post cardiac arrest syndrome (PCAS) develop severe hemostatic aberrations and coagulopathy, which are associated with excess mortality that has not improved for decades. Despite the different types of injurious "hit", acutely critically ill patients share several phenotypic features that may be driven by the shock. This response, mounted by the body to various life-threatening conditions, is relatively homogenous and evolutionarily adapted. We propose that shock-induced sympathoadrenal hyperactivation is a critical driver of endothelial cell and glycocalyx damage(endotheliopathy) in acute critical illness, with the overall aim of ensuring organ perfusion through an injured microvasculature. We have investigated more than 3000 patients suffering from different types of acute critical illness (severe trauma, sepsis, MI and PCAS) and have found a unifying pathophysiological link between sympathoadrenal hyperactivation, endotheliopathy, and poor outcome. We entitled this disease entity, shock induced endotheliopathy (SHINE).

3C-S14-02

BLOOD COMPONENTS – SO MUCH MORE THAN CLOTS AND OXYGEN DELIVERY!

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Blood, and the blood components packed red blood cells (pRBC), platelet concentrate (PC), plasma and cryoprecipitate, has traditionally been transfused to maintain or improve oxygen delivery, blood volume and/or hemostatic capacity. There is however emerging evidence that blood components may support other alternative functions beyond oxygen delivery, volume and hemostasis. Thus, PC may through their functions as innate immunologic effector cells improve immune competence. Plasma may through its endothelial protective and repairing function improve vascular integrity and hence outcome in critical illness. Finally, erythrocytes and thus pRBC may be critical regulators of the vascular, hemostatic and immune systems through their hemostatic function, their chemokine scavenging capacity and their endothelial cross talk functions. The present review reveals and discusses some emerging new functions of blood and blood components and thus alternative indications for transfusion.

3C-S14-03

DIFFERENCES IN RED CELL TRANSFUSION DECISIONS IN THE DUTCH INTENSIVE CARE UNIT

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Background: Although several studies have demonstrated the safety of restrictive transfusion triggers of less than 7 g/dl in critically ill patients, high-quality evidence for individual transfusion triggers is still lacking. Recent guidelines therefore recommend to take patient characteristics into account when deciding whether or not to transfuse. The lack of high-quality evidence together with unclear recommendations can lead to variation in transfusion practice.
Aims: The primary objective of this study was to uncover variation in transfusion decisions in four different clinical scenarios of critically ill patients among intensive care physicians in the Netherlands. Secondary objectives included the assessment of physicians' perceptions of transfusion risks and benefits and the influence of these on their transfusion decision.
Methods: An online survey comprising four different clinical scenario's was sent to all 1011 members of the Dutch Society of Intensive Care. The scenarios represented patients with an acute myocardial infarction (AMI) (Hb 7.3 g/dl), abdominal sepsis (Hb 7.1 g/dl), traumatic brain injury (TBI) (Hb 7.9 g/dl) and postsurgical complications after gastro-intestinal surgery (Hb 7.3 g/dl). The 4 questions in each scenario explored the decision whether or not to transfuse and the perceived likelihood of benefits (based on the probability of preventing ischemic organ damage) and harms (based on the probability of TACO/TRALI) of transfusion.
The distribution of the transfusion decisions was explored for each clinical scenario. We used Generalized Estimated Equations (GEE) to analyze the association between the perceived likelihood of benefits and harms and the participants' transfusion decision as dependent variables, taking into account clustering within respondents, and adjusted for clinical scenario. Additionally, we calculated the benefit-harm ratio for

each participant (perceived probability of benefits divided by the perceived probability of harms) to explore the association between this ratio and the willingness to transfuse.
Results: A total of 227 (22%) members participated in the study, of whom 182 (80%) completed all questions. In the case of the patient with AMI 74.8% of the respondents decided to transfuse. In the patients with sepsis, TBI and postsurgical complications respectively 43.1%, 26.2% and 81.5% of the respondents decided to transfuse. The GEE showed that the odds of transfusion was 2.57 times higher for every 10% increase in perceived probability of benefit (OR 2.57; 95% C.I. 2.14 – 3.08) and 0.68 times lower for each 10% increase in perceived probability of harm (OR 0.68; 95% C.I. 0.56 – 0.84). The distribution of the decision to transfuse across different strata of the individual benefit-harm ratio was as follows: <0.3: 1%; 0.3–0.9: 23%; 0.9-11: 24%; 1.1-3: 74%; >3: 85%.
Summary / Conclusions: Physicians decided differently about red blood cell transfusion given the clinical scenarios. As shown by the variation in distributions between the clinical scenarios, it is likely that physicians weigh clinical variables differently in their transfusion decision.
Secondly, our study showed independent associations between perceived probabilities of benefit and harm and the decision to transfuse. This study also showed that physicians tend not to transfuse when perceived benefits and risks are about equal (benefit-harm ratio = 1).

3C-S14-04

ORAL ANTICOAGULANT AGENT-ASSOCIATED BLEEDING EVENTS REPORTING SYSTEM (ORANGE) STUDY

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Background: Major bleeding is a recognised complication of oral anticoagulant (OAC) therapy, but there is little information on its associated outcomes between different OAC agents.
Aims: To compare the burden of major bleeding between warfarin and direct oral anticoagulants (DOACs) in the UK and to identify risk factors for death.
Methods: UK multicentre prospective observational study that collected data on major bleeding cases from 32 hospitals between October 2013 and September 2016.
Results: 2192 patients (53% male), median (interquartile range, IQR) age of 80 (72–86) years were reported. Of these 81%, 13%, 4%, 2% and 0.1% were due to warfarin, rivaroxaban, apixaban, dabigatran and edoxaban respectively. The proportions of patients with an intracranial haemorrhage (ICH), gastro-intestinal (GI) bleed, and others were 44%, 33% and 24% respectively. Comparing warfarin and DOAC, the former had a greater proportion of subdural/epidural bleeds (21% vs. 12%) but a lower proportion of GI bleeds (30% vs. 44%). For warfarin patients, 74%, 78% and 34% received vitamin K, prothrombin-complex-concentrate (PCC) and blood transfusion (includes any blood components) respectively. For DOAC patients, 28%, 41% and 46% received tranexamic acid, PCC/Factor-Eight-Inhibitor-Bypass-Activity, and blood transfusion respectively. The overall 30-day mortality rate was 20% (95 CI 18.6-22) and the median (IQR) stay in hospital for those who survived was 7 (3-13) days. There was no difference in mortality and hospitalisation between different OACs, and logistic regression (n = 2132) showed no evidence that OAC type (P = 0.99) or indications (all P > 0.34) were independently associated with mortality. The main risk factors for death were ICH, advanced age, spontaneous bleeding, liver failure and cancer.
Summary / Conclusions: there was no difference in the burden of major bleeding associated with different OAC in terms of mortality and hospitalisation.

Resource Limited Countries: Haemoglobinopathies in the developing world

3C-S15-01

PRACTICAL ASPECTS OF SICKLE CELL DISEASE

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Sickle cell disease is the commonest genetic disease in man with the highest prevalence in Nigeria, Democratic Republic of Congo and the India subcontinent. In affected individuals, the illness manifests early in life and progresses with periods of relative good health punctuated by catastrophic illness and bone pains. The clinical course is variable with complications such as heart disease, stroke, hypertension, renal failure and others occurring with increasing age and affecting almost every organ system in the body.
Diagnosis by electrophoresis, iso-electric focusing, high performance liquid chromatography, mass spectrometry, new born screening (NBS) or molecular methods have varying sensitivities, cost and availability. NBS, followed by evidence based interventions, improves outcome in children with SCD but is yet to be implemented in developing countries due to poor infrastructure and inadequate resources. These challenges have prompted the development of point-of-care testing devises which require no electricity and can be used by front line health care workers to screen for SCD.
The curative treatment for SCD is stem cell transplantation which is limited by cost, availability of HLA matched donors, complications such as graft rejection, graft vs host disease (GVHD), appropriate infrastructure and skilled manpower to support such treatment. Autologous bone marrow transplant of genome edited $CD34^+$ haematopoietic stem and progenitor cells and use of reduced intensity conditioning regimen seek to reduce GVHD. Recent trials have explored treatment with lentiviral vector -mediated addition of an anti-sickling β-globin gene into autologous haematopoietic stem cells with success in SCD with no recurrence of crises in SCD, correction of biological hallmarks of SCD and consistent safety profiles with no evidence of insertional mutagenes. Also recently, an antibody to P-selectin produced significantly lower rate of sickle pain crisis compared with placebo and was associated with a low incidence of adverse events in a phase II trial.
Blood transfusion is not required at steady state haemoglobin in SCD but in the management of acute chest syndrome, (ACS), primary and secondary stroke prevention and in preparation for surgery. In countries with poorly developed transfusion services and high prevalence of transfusion transmissible viruses, chronic transfusion for primary and secondary stroke prevention is not feasible but hydroxyurea treatment in SCD children with high risk of stroke has shown promise in recent trials in such settings.
Health maintenance for patients with SCD with prophylaxis for infection, adequate nutrition and hydration, prompt treatment of crisis and complications is beneficial and should be available in primary care. Health workers should be trained to use evidence based guidelines in the management of acute and chronic complications of SCD and to deliver comprehensive care services.
However, as the quest for improved universal access to screening,diagnostic and comprehensive care services and safe and accessible curative treatment for SCD continues, patients feel that for a 100% preventable disease, they would much rather not have to deal with it in the first place. Therefore effort should be made to explore public health and culturally acceptable ways to reduce the number of sickle cell births.

3C-S15-02

NEWBORN SCREENING FOR SICKLE CELL DISEASE IN GHANA- 20 YEARS OF TESTING, TRACKING AND FOLLOW-UP

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Introduction: Newborn screening (NBS) for Sickle Cell Disease (SCD) is designed to identify newborns who have the disease early so that, they can be started on penicillin prophylaxis and enrolled into a comprehensive clinical care programme. We present the results of two decades of NBS for SCD in Ghana.
Objective: To assess the programme of screening and follow-up of newborns with SCD in Ghana.
Methods: Blood sample is taken from a heel prick of the baby onto a filter paper sample form; the forms are shipped to the newborn screening laboratory at Noguchi Memorial Institute for Medical Research, where the dried blood spots are eluted and used for identification of haemoglobin variants by Isoelectric Focusing (IEF) method. These samples are taken at birth or within days or few weeks after birth when the babies visit the Well-infant Clinics for health assessment and immunization. Currently, screening of newborns occurs at 37 public and private facilities in Kumasi, the 2nd largest city in Ghana, and at 1 private midwifery clinic in a rural town-Tikrom. At the time of screening, mothers are informed to return to the point of screening for results within 4 weeks. If a mother with an infant with Possible SCD (P-SCD) does not return for the results, community health nurses visit the home (using information gathered at the time of screening) to deliver results. Mothers who have telephones are called to return to receive the test result. Newborns identified with SCD are enrolled by eight weeks when they begin to receive twice daily penicillin and comprehensive care at the Kumasi Centre for Sickle Cell Disease (KC-SCD) at Komfo Anokye Teaching Hospital (Kumasi, Ghana). Data from all aspects of the NBS programme are managed through two databases – one tracking logistical activities at the screening sites and the other tracking data about each baby screened.
Health education programmes are also set up to educate pregnant women, parents of children with SCD, and the general public, about the disease and the NBS programme.
Results: As of December 31st, 2015, a total of 455,483 infants had been screened; 7,798 (1.7%) with haemoglobin (Hb) phenotypes - FS, 4016 (0.88%), FSC, 3,708 (0.81%), and, FSA, 74 (0.02%) were identified to have SCD.
Of the 7,798 infants with possible SCD, 6,160 (79.0%) have been successfully tracked and the families contacted. Of these, 156 had died before the families were located. Of the 6,004 available for clinic enrollment 5,683 (94.7%) have so far been enrolled.
Conclusions: Two decades of Screening and follow-up of newborns for SCD in a resource-limited country in Africa, has been largely successful. However, challenges still exist in tracking of babies with SCD and extra effort and innovative ways are needed to ensure that those infants are found early and referred for medical management.
Key words: Haemoglobin, Newborn Screening, Sickle Cell Disease, Tracking.

3C-S15-03

NONGENETIC MODIFIERS OF DISEASE SEVERITY IN SCD

F Piel

No Abstract available

Parallel sessions

Donors/Donation: Plasma supply management

4A-S16-01

SAFE AND SUSTAINABLE PLASMAPHERESIS

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Background: Australian immunoglobulin demand has grown at an average of 12% p.a. for the last 10 years. The reduction in demand for red cells has reduced the availability of recovered plasma and has increased the reliance on plasmapheresis collections. In 2015/16 more than 601 tonne of plasma for fractionation was collected by the Australian Red Cross Blood Service within an entirely voluntary, non-remunerated blood donor system, with 71% sourced from plasmapheresis collections. Saline compensation (500 ml) is used for all plasmapheresis collections by 2 different protocols, mid-donation for plasma for fractionation (stepped regimen up to 18% of estimated TBV with a maximum of 800 ml) and end-donation intended for clinical plasma (stepped regimen up to 16% of estimated TBV with a maximum of 750 ml). The plasmapheresis panel includes both new and very experienced donors. More than 350 donors have each given more than 350 donations. Several donors have given more than 1,000 donations. Whilst blood donation is generally a safe process, there are recognised donor complications which can cause donor harm. Donor discomfort, whilst not causing donor harm per se, impacts program sustainability by reducing donor return rates. A simple electronic donor vigilance reporting system supports the capture of immediate adverse events. Donors are also specifically questioned about delayed adverse events at each subsequent donation.
Material and Methods: IgG levels in 5058 first time whole blood Australian donors were measured to determine a normal range and the impact of age. An analysis was performed to determine the effect of age and donation frequency on IgG levels in plasmapheresis donors. The impact of different collection protocols on both the amount of IgG in the bag and the concentration of IgG was determined. Donor adverse event rates for both male and female donors at all TBV collection limits for both mid-donation and end-donation protocols were analysed.
Results: IgG levels fall with aging, particularly after 50 years of age. As a result, donor age can influence IgG yield. As the volume of plasma collected and procedure duration increases, the IgG concentration drops. Mid-donation saline compensation will further reduce IgG concentration and this may not be fully compensated by the increased collection volume permitted by our protocol. Recovered plasma has the highest IgG concentration, but the lowest amount of IgG per collection. Many factors affect whether a donor faints. Whilst the adverse event rate for end-saline 16% TBV collections was statistically significantly greater compared with mid-saline 18% TBV collections, both were significantly lower than the whole blood donation event rate. The adverse event rate for both males and females increases as the actual percentage of TBV collected increases. Females have a higher relative risk of donor adverse events compared with males.
Conclusions: Long term donors in Australian moderate intensity plasmapheresis programs maintain IgG levels within normal ranges over decades. The collection protocol significantly impacts both IgG concentration, the amount of IgG in the collection bag and donor adverse event rates. Relatively minor changes in donor demographics can significantly impact IgG yield and donor adverse event rates.

4A-S16-02

SELF-SUFFICIENCY IN PLASMA SUPPLY

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In May 2010 the World Health Assembly passed a resolution, WHA 63.12, on the availability, safety and quality of blood products. This urges member states to 'take all necessary steps to establish, implement and support nationally coordinated BTS with the aim of achieving self-sufficiency". The resolution clearly links the goal of self-sufficiency to the principle of voluntary non-remunerated donation (VNRD). It applies to both fresh blood components and Plasma Derived Medicinal Products (PDMPs). WHO defines self-sufficiency as meeting national needs for six products

from blood and plasma collected locally. These comprise red cells, platelet concentrates, fresh frozen plasma, and Factor VIII, immunoglobulin and albumin solution. The definition and overall goals were established by a WHO Expert Consensus Statement on 'Achieving Self-Sufficiency Based on VNRD' developed at a meeting of WHO experts in Geneva Switzerland in 2011.

Blood Services develop in line with the health care system that they support. Typically this involves a transition from a supply for whole blood mainly in the context of emergency management of trauma and child birth to the implementation of blood component therapy. Blood component production will likely lead to an excess of recovered plasma. Effective utilisation of this requires access to plasma fractionation facilities. If this can be achieved then this will reduce reliance on commercial PDMPs and ensure maximal utilisation of the donated whole blood. Increasing demand for PDMPs will potentially lead to a requirement for introduction of plasmapheresis programmes if the goal of self-sufficiency is to be realised.

There are a number of barriers to both utilisation of recovered plasma and the implementation of effective plasmapheresis programmes. Individual countries will need to consider these as part of their long term strategic planning process. The main barrier for low and medium HDI index countries is access to suitable plasma fractionation facilities. Few countries will be able to justify creation of a national fractionation plant. Regional co-operation and development of facilities may be needed. Secondly plasma for fractionation is seen as a critical material in the manufacture of a medicine. Quality system requirements, including full compliance with a code of Good Manufacturing Practice will be a pre-requisite for acceptance of the plasma as suitable for fractionation. There is an urgent need to develop support programmes to raise standards to achieve this. In contrast the main barrier for most high HDI countries is cost and the easy availability of PDMPs produced from paid plasma. The safety profile of PDMPs is currently excellent since the integration of a number of specific viral inactivation steps in the manufacturing process overcomes any increased infectious burden associated with payment for plasma. The critical question is then how a country can assure ongoing supply at a reasonable cost. Ethical principles will play an important role in defining the strategy and individual countries should be able to define their own approach without unnecessary intervention and competition from the commercial plasma industry.

4A-S16-03
THE FUTURE OF PLASMA COLLECTION

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Plasma-derived medicinal products (PDMPs) such as the clotting factors VIII and IX, polyvalent immunoglobulins and hyper-immune globulins are listed as Essential Medicines by the World Health Organisation. These and other PDMPs are crucial for the prophylaxis and treatment of patients with bleeding disorders, immune deficiencies and auto-immune and inflammatory diseases, and a variety of congenital deficiency disorders. However the supply of plasma required to manufacture these medicines is limiting and in danger of disruption. Changing medical practices have reduced red cell transfusions and the whole blood collections from which "recovered plasma" for fractionation derives. This trend is predicted to continue. Relatively few commercial plasma collectors, located primarily in the United States (US), provide an increasing percentage of the world's source material for fractionation. In 2014, source plasma was collected by 552 commercial plasmapheresis centers of which 80% are located in the US. Sixty percent of this plasma originates from US paid donors representing the majority contribution to the worldwide supply. Contributions from other regions, and in particular from Europe, are considerably lower. Reliance on any single region to supply the majority of the world's plasma raises several concerns. Several situations might lead to future plasma supply interruptions. Regional plasma shortages may arise from the emergence of unexpected transfusion-transmissible infections (TTIs), economic factors, commercial consolidation, changes in demand elsewhere, and the flow of medicines from countries with lower prices to countries with higher prices. Epidemics of transfusion-transmitted infectious agents as have happened by the unexpected appearance of Human Immunedeficiency Virus in the 1980s, variant Creutzfeldt Jakob Disease in the United Kingdom and Ireland, Chikungunya virus and more recently in the US territory of Puerto Rico by epidemics of Dengue and Zika viruses can threaten regional blood and plasma collections and endanger global supplies of PDMPs. Plasma for fractionation should therefore be considered a "strategic resource." Strategic resources are defined as "economically important raw materials which are subject to a higher risk of supply interruption". What makes these materials critical for a region or a country is the lack of domestic production and/or inability to guarantee national supply through importation. National industries can become completely dependent on importation, particularly during periods of expanding market demand. Plasma fits the definition of a "strategic resource." We submit that plasma should be considered not only a precious human resource, but also a strategic one. We advocate the need for improved and equitable balance of the international plasma supply in order to reduce the risk of supply shortages worldwide. We propose that blood and plasma should be considered strategic resources. The aim is to highlight the importance of balanced and diverse collection and preparation, and to minimize the risk of shortages of plasma and essential PDMPs upon which patients' lives depend. Steps can be taken in both developed and developing countries to safeguard the plasma supplies that are necessary to prevent regional and global shortages of essential PDMPs.

Reference: Strengers, Paul F.W., and Klein, Harvey G. Plasma is a Strategic Resource. Transfusion. 2016; 56:3133–3137.

Immunobiology of blood cells: Fetomaternal immunology

4A-S17-01
ANTENATAL DETERMINATION OF FETAL BLOOD GROUPS OR ANTIGENS

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During pregnancy, alloantibodies directed against blood group antigens on red blood cells or platelets mean a putative danger to the health of the fetus. In pregnancy there is an active transport of IgG-class immunoglobulins from the mother to her unborn child. Among the protective IgG immunoglobulins also blood cell destructing IgG alloantibodies may be present. These can bind to the fetal blood cells if the fetus is positive for the involved blood group antigen. Non-invasive prenatal genetic blood group typing has been a long-sought-after goal in prenatal medicine, avoiding the around 1% risk of miscarriage associated with invasive procedures. Since the start of this century there is sufficient evidence published that placenta-derived cell-free DNA present in maternal plasma can be used for reliable fetal blood group antigen typing. There are several challenges for accurate fetal blood group antigen genotyping. First the absolute quantity of fetal cell-free DNA is low, and depends on duration of pregnancy but also shows a wide range. Second the relative quantity of fetal cell-free fetal DNA. False positive signals may be generated from maternal DNA sequences and test accuracy may be influenced depending on the used genotyping platform. Third, the design of the genotyping assays may be hampered by the short length of the fetal DNA fragments. And fourth, genetic variation, sometimes ethnicity dependent, may influence the correct prediction of the fetal phenotype. Both by real-time quantitative PCR, droplet digital PCR or targeted massively parallel sequencing these challenges can be met. In many countries non-invasive fetal blood group antigen genotyping is part of the laboratory work up of alloimmunized women. Knowledge on the fetal blood group type in those pregnancies prevents unnecessary clinical follow-up and interventions. It facilitates optimal selection of high-risk pregnancies. And it may lower anxiety of the parents. It is a long awaited improvement in a value-based health care system. Another example of such an improvement is the use of fetal *RHD* genotyping to target the antenatal and postnatal anti-D immunoglobulin prophylaxis to RhD-negative women. The use of fetal *RHD* genotyping prevents the unnecessary administration of anti-D immunoglobulin and may increase efficiency because postnatal cord blood RhD serology can be stopped. Evaluating risks (e.g. RhD immunization due to false-negative fetal *RHD* typing results), benefits (e.g. reduction in use of anti-D immunoglobulin) vs costs and cost reductions, lead to different considerations in various countries and influence the decision on implementation fetal *RHD* typing in anti-D prophylaxis programs.

4A-S17-02

ANTIENDOTHELIAL ALPHAVBETA3 ANTIBODIES AS A MAJOR CAUSE OF INTRACRANIAL BLEEDING IN FNAIT

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Fetal/neonatal alloimmune thrombocytopenia (FNAIT) is a severe bleeding disorder, which can result in intracranial hemorrhage (ICH), leading to death or neurological sequelae. In Caucasians, maternal anti-HPA-1a antibodies (abs) are responsible for the majority of FNAIT cases. These antibodies can eliminate circulating fetal platelets. However, the correlation between fetal platelet counts and risk of bleeding is loose: very low platelet counts are frequently found in fetuses without any bleeding, and ICH has been reported in FNAIT cases where the platelet counts were within reference ranges. These observations question the exclusive role of thrombocytopenia in ICH.

By comparing sera derived from mothers with ICH-positive (+ICH) and ICH-negative (-ICH) FNAIT cases, we could recently observe stronger binding of +ICH IgG to endothelial cell (EC)-derived αvβ3. Differential absorption experiments demonstrated that anti-HPA-1a is not a single antibody entity. Sera from immunized women may contain up to three different sub-specificities: anti-β3, anti-αIIbβ3, and anti-αvβ3. Anti-β3 appears to be the dominant subtype of anti-HPA-1a in most women. In contrast, the anti-αvβ3 subtype was only observed in +ICH sera. In addition, only the anti-αvβ3 subtype, but not anti-β3 subtype, induced EC apoptosis, interfered with EC-adhesion to vitronectin, and disturbed EC tube formation in vitro. A modified human monoclonal antibody against HPA-1a with anti-β3 specificity (moab deg-813) did not cause endothelial disturbance, but inhibited anti-endothelial effects of anti-HPA-1a of the αvβ3 subtype. IVIG mediates similar protective effects in vitro.

Since anti-αvβ3 was identified in the +ICH cohort and mediates significant anti-endothelial activity, we believe that this anti-HPA-1a subtype plays a significant role in the development of ICH. Anti-αvβ3 and other subtypes of anti-HPA-1a may probably act together in a threshold model of FNAIT/ICH, where bleeding occurs if yet undefined levels of anti-endothelial (loss of integrity) and/or anti-platelet (low platelet count) activity are achieved. Defining anti-HPA-1a subtypes in maternal serum may have significant potential in the diagnostic prediction of ICH development. It may also allow for modification of prophylactic treatment. In vitro, IVIG and deg-813 are protective against the anti-endothelial activity of anti-αvβ3. With upcoming nation-wide screening programmes, detection of anti-HPA-1a subtypes could prove helpful in avoiding overtreatment. It may also help in allocating treatment to appropriate (at-risk) cases in countries with restricted resources.

4A-S17-03

ANTI-CD36 ANTIBODIES IMPAIR THE FUNCTION OF MICROVASCULAR RATHER THAN MACROVASCULAR ENDOTHELIAL CELLS: MECHANISM OF HYDROPS?

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Background: Anti-CD36 is the most frequent platelet antibody (ab) in fetal and neonatal alloimmune thrombocytopenia (FNAIT) in Asians. Anit-CD36 (also known as anti-Nak[a]) developed by CD36 deficient (CD36*null*) mothers passing the placenta and binding to fetal platelets are assumed to be responsible for these FNAIT cases. The clinical picture of anti-CD36 mediated FNAIT, however, is heterogeneous, ranging from thrombocytopenia with widespread petechiae, gastrointestinal bleeding, to severe anaemia and fetal hydrops. Among these, fetal hydrops is the most frequent consequence of anti-CD36 mediated FNAIT. The mechanism of this phenomenon, however, is unclear. It is known that in addition to platelets, CD36 is also expressed on monocytes, macrophages, erythroid precursors and endothelial cells. Recent studies from our group showed that antibody bound to HPA-1a epitopes residing on avb3 integrin of endothelial cells alter endothelial dysfunction; a mechanism associated with the development of intracranial haemorrhage (Santoso et al, 2016).

Aims: In this study, we ask the question whether anti-CD36 may also directly alter endothelial function.

Methods: The effect of anti-CD36 antibodies on endothelial permeability (fluorescence labelled BSA), apoptosis (Caspase3/7 assay) and angiogenesis (tube formation assay) were investigated.

Results: Treatment of human macrovascular endothelial cells (HUVEC) with anti-CD36 abs for 12 h caused significant increase of albumin flux through an endothelial monolayer. Interestingly, this phenomenon was not observed with anti-HPA-1a abs. Anti-CD36 mediated permeability was more pronounced when human microvascular endothelial cells (HMVEC) were investigated. This is in accordance with our finding that HMVEC express CD36 with higher density than HUVEC. Furthermore, the influence of anti-CD36 abs on endothelial apoptosis and endothelial tube formation was analysed under similar conditions. Anti-CD36 abs induced apoptosis and impaired tube formation of HMVEC, but not HUVEC. In comparison to anti-HPA-1a abs, however, the effect was significantly lower.

Summary / Conclusions: In conclusion, our observation indicates that anti-CD36 abs impair the function of microvascular rather than macrovascular endothelial cells. In contrast to anti-HPA-1a abs, anti-CD36 abs affects endothelial permeability rather than vessel formation. Our finding suggests a direct involvement of anti-CD36 abs in endothelial barrier dysregulation, which appears to constitute a central pathway in the development of fetal hydrops in CD36-mediated FNAIT.

4A-S17-04

PREDICTION OF FETAL HPA-1A OR OTHER BLOOD GROUP ANTIGENS FROM MATERNAL PLASMA USING NEXT GENERATION SEQUENCING

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Background: Anti-HPA-1a antibodies produced by HPA-1a neg pregnant women can cause fetal/neonatal alloimmune thrombocytopenia (FNAIT). The prediction of the fetus HPA-1a status is important for decision concerning management of pregnancy. The cell-free fetal DNA is widely used for determination of fetal blood groups but obtaining of proper specificity in the real-time PCR of a single nucleotide polymorphism (SNP), such as HPA-1a, requires modified protocols. Next generation sequencing (NGS) enables the high coverage sequencing of target SNP position and is an important alternative for detection of low-grade fetal chimerism in maternal plasma DNA.

Aims: To establish NGS protocol for non-invasive prenatal diagnostics (NIPD) of *HPA-1a* and other clinically important blood group antigens.

Methods: DNA was isolated from plasma samples of 4 donors and 5 pregnant women with known genotypes (easyMag, Biomerieux). Using Ion AmpliSeq Designer we designed specific primers for sequencing RhD, RhC/c, RhE/e and primers flanking SNPs regions for K/k, Fya/b, Jka/b, MN, Ss, HPA-1, 2, 3, 5, 15 regions and 4 X-chromosome SNPs. PCR products were sequenced using Ion Torrent PGM (Thermo).

Results: The results of NGS were concordant with known phenotype/genotype of donors, pregnant women and newborns. The NGS protocol for NIPD detected feto-maternal incompatibility in HPA-1a, -2b, -3a, -5a, -15a, K, Fya, Fyb and in 2/4 X-chromosome SNPs (fetal chimerism from 1.1% to 4.1%; unspecific reads below 0.56%) and in RhD, Rhc (fetal chimerism 100%; no signal form maternal DNA). Detection of MN alleles using designed primers failed.

Summary / Conclusions: The NGS technology enables the prediction of fetal HPA-1a as well as others clinically important platelet and erythrocyte antigens. Further optimization of primers for MN alleles is needed.

Blood Safety: Risk management

4A-S18-01

US BLOOD DONORS WITH EVIDENCE OF ZIKA VIRUS INFECTION OUTSIDE AREAS OF ACTIVE TRANSMISSION

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Background: Zika virus (ZIKV) is transmitted by *Aedes* mosquitos and can result in severe congenital and adult neurological abnormalities. A minority of infected persons experience symptoms and relatively high rates of viremia were detected in blood donors during prior ZIKV epidemics in French Polynesia and Caribbean islands. ZIKV has rapidly spread northward through Central America and the Caribbean in 2015 and autochthonous cases have been identified in the continental US in 2016. The potential for ZIKV transmission by transfused blood from healthy donor components has been a growing concern. As a result, the US FDA issued guidance in August 2016 which recommended implementation of individual donation NAT (ID-NAT) for the entire US by November 2016.

Aims: To describe the rate of ZIKV RNA positive blood donations in the continental US in areas without active ZIKV transmission.

Methods: Individual donation aliquots of plasma from volunteer blood donors were tested under an IND clinical protocol with the investigational Procleix Zika Virus Assay based on target-capture transcription-mediated-amplification (TC-TMA; Hologic/Grifols) results. Initially reactive samples were further tested for ZIKV RNA in plasma and RBCs by PCR and for ZIKV-specific antibodies by IgM and IgG ELISAs and Reporter Viral Particle Neutralization (RVPN) testing and donors were enrolled into follow up studies with repeat TC-TMA and supplemental ZIKV testing. A confirmed positive classification required confirmation of RNA and/or detection of ZIKV antibodies in index and/or follow-up samples.

Results: From September 19, 2016 to January 31, 2017, 933,831 donations in US states were screened for ZIKV RNA by Creative Testing Solutions using the Hologic/Grifols investigational Procleix Zika Virus Assay. Thirty two donors were initially reactive, of which, ten (1 in 107,000) were confirmed as probable for prior ZIKV infection. Viral RNA concentrations were extremely low in plasma but readily detected in RBC samples. All confirmed donations were collected outside areas considered to have active transmission, with the exception of a single south Texas case. A high proportion of the confirmed donors had travel exposures in areas with active ZIKV transmission ranging from ~1 to 3 months (34 to 97 days) prior to donation. Although these donors were infected several months prior to the index donations, they continued to demonstrate RNA associated with RBC for 2–3 months post-exposure and steady or increasing ZIKV IgG reactivity by ELISA and ZIKV-specific neutralizing activity by RVPN testing, with declining ZIKV IgM reactivity.

Summary / Conclusions: This report documents moderate rates of ZIKV positive blood donors outside areas with active transmission (Puerto Rico, Florida and Texas). These donors most likely represent travel-acquired "tail-end infections" with prolonged RBC-associated ZIKV RNA.

4A-S18-02

BLOOD PRODUCT USAGE DURING PREGNANCY IN DENMARK AND SWEDEN AND ITS RELEVANCE FOR ZIKA VIRUS RISK ASSESSMENT

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Background: Although the course is most often asymptomatic or mild, Zika virus infection during pregnancy may cause microcephaly or other severe congenital neurologic malformations in the foetus. Probable cases of Zika virus infection through blood transfusion have been reported sporadically. To reduce the risk of transmission through transfusion blood donors are temporarily deferred after travel to areas with Zika virus transmission, a means that is most often preferred above a costly donation screening for Zika virus RNA. With the discovery of sexual transmission of Zika virus infection we face complex algorithms for deferral and risk assessments have emerged to evaluate the importance of this route of infection for blood safety. In such assessments, it is often assumed that 1% of blood products are transfused to pregnant women.

Aims: To evaluate the proportion of blood products transfused to pregnant women by means of a bi-national database covering all transfusions and pregnancies.

Methods: The Scandinavian Donations and Transfusions database (SCANDAT) comprises data from blood donations and transfusions administered to recipients in Denmark and Sweden. All blood products (red blood cell units, fresh frozen or cryo-enriched plasma, and platelets) transfused during pregnancy in Denmark (2000–2008) or Sweden (2000–2010) were analyzed.

Results: During the study period, 11,525,301 products (36% in Denmark) were transfused in Denmark and Sweden. In the same period, 1,626,661 women gave birth in the two countries. Of the Danish and Swedish women, 0.03% and 0.04%, respectively, had been transfused during the first two trimesters of pregnancy, and 0.13% and 0.14%, respectively, at least once sometime during pregnancy. The transfused women received a total of 9,181 products, in Denmark corresponding to 54/100,000 transfused products and in Sweden to 94/100,000 transfused products.

In both countries, most of the transfused products were given during the third trimester, while transfusions earlier in pregnancy accounted for 16/100,000 and 26/100,000 products in Denmark and Sweden, respectively. The number of products transfused per pregnancy was lower in Denmark (3.8 products/1000 pregnancies; 1.2 products/1000 pregnancies in the first and second trimester) than in Sweden (6.7 products/1000 pregnancies; 1.8 products/1000 pregnancies in the first and second trimester).

Summary / Conclusions: The fraction of blood products used for pregnant women was low, accounting for only 54 and 94 per 100,000 blood products overall. Most transfusions were given in the third trimester of pregnancy, during which risk of Zika induced foetal malformation most likely is the lowest. The estimated risk of sexually acquired Zika virus infection in donors in non-endemic countries and the risk of a viremic donation is very low. This study supports that the risk of transferring Zika virus to pregnant women by blood transfusion is lower than often assumed. Blood usage in pregnancy may differ significantly between countries as is also seen for Denmark and Sweden. The reason for the higher blood usage in pregnancy in Sweden is currently under investigation.

4A-S18-03

RARE DONORS AND MALARIA SEMI-IMMUNITY

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Background: Migratory flows of sub-saharan (SSA) persons throughout the world are expected to continuously increase. A significant proportion of SSA citizens are affected by Sickle Cell Disease (SCD), condition requiring repeated blood transfusions. Many centuries of malaria pressure have induced in SSA natives a homogeneous selection of peculiar haematologic characteristics, such as the absence of high

frequency red cell antigens (defining a rare blood) that cannot be found in donors of European descent. Unmatched transfusions are the main reason why many SCD transfused patients experience the fearful occurrence of red cell alloimmunization. For the aforementioned reasons haematologists are expecting to access to Rare Blood Banks in order to assure a full match between donor and recipient's blood, that may be obtained from donors sharing the same ethnicity. Unfortunately SSA donor recruitment is counteracted by the widespread diffusion of infections contracted before migration: one of these is malaria. In SSA malaria may occur subclinically and is characterized by a slow antibody clearance. This peculiar condition, the so-called semi-immunity, is a kind of co-evolutionary process characterized by the co-existence and persistence of small entity of *Plasmodium* genome and relative antibodies. Molecular techniques are unreliable to detect a small number of Plasmodia, which may otherwise be sufficient to induce a transfusion transmitted malaria (TTM). The serologic assessment, despite the low specificity, remains the most sensitive and reliable method to detect the semi-immune status in blood donors (1).

Aims: The aim of this study was to assess the prevalence of malaria immunity in a cohort of healthy SSA citizens.

Methods: Since 2010 in our Department of Haematology and Transfusion Medicine we recruited 184 SSA citizens, in good health, who agreed to underwent clinical and laboratory investigations to become a blood donor. All of them were born in SSA Africa and lived there for at least the first 5 years of life. 70% of subjects didn't recognize any previous malaria fever. The last travel/stay in Africa was 1–20 years (median 3 years), and 48% of returning people had received prophylaxis. Malaria serology was determined by a commercial enzyme immunoassay kit (Malaria EIA Ab, Bio-Rad).

Results: Overall 75% of persons were positive for malaria antibodies. Serologic positivity was found in 75% of persons no more exposed in 5 recent years and even in 83% (19/23) persons settled in Italy since 10–20 years. Serologic positivity was present in 100% of people from Benin, 85% from Burkina Faso, 78% from Ivory Coast and Cameroon, 63% from Senegal. We followed antibody concentration in 50 persons (136 assays), and we observed a slightly negative trend that, in most cases, was followed by a prolonged phase of low antibody levels. 4/50 became negative after 3 years.

Summary / Conclusions: The identification of malaria antibodies is essential in SSA native donors and, by far, irreplaceable in order to avoid the risk of TTM. Until pathogen inactivation techniques will become available, we have a very low expectation to introduce SSA blood in Blood Bank inventories. Haematologists have to wait some years for the forthcoming SSA second generation that will allow to fully match the entire SCD patient community. 1- Assennato SM, Berzuini A *et al*. Transfusion 2014 https://doi.org/10.1111/trf.12650.

4A-S18-04

RESIDUAL RISK OF BACTERIAL CONTAMINATION OF PLATELET CONCENTRATES: SIX YEARS OF EXPERIENCE WITH STERILITY TESTING AT CANADIAN BLOOD SERVICES

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Background: At Canadian Blood Services, production of platelet concentrates comprises approximately 70% of four-unit buffy coat pools and 30% single-donor apheresis units. All platelet products are leukocyte-reduced and screened for bacterial contamination using aerobic BacT/ALERT culture bottles within 24–30 h post-phlebotomy. Missed bacterial detection of contaminated platelet concentrates during early routine culture screening still occurs with the risk of being released into inventory. Contaminated platelet units with negative screen results may be found through reporting of transfusion reactions or through repeat testing of platelet components at outdate. Canadian Blood Services performs Quality Control (QC) sterility testing of 1% outdated platelet concentrates (or minimum 10 units) on a monthly basis. QC testing is performed in 6-7 day-old apheresis and buffy coat platelets using aerobic and anaerobic BacT/ALERT bottles. Reports of transfusion septic reactions are documented by Regulatory Affairs.

Aims: This report was aimed at investigating the residual risk of bacterial contamination in platelet concentrates at Canadian Blood Services. National data of routine screening, QC testing, and septic reactions, gathered from 2010 to 2016, are presented in this report.

Methods: From 2010 to 2016, 601,988 buffy coat pools and 186,737 apheresis units were screened during routine testing. In the same period, 8,535 pools and 8,498 apheresis platelets were screened during QC testing. Positive results were classified as: "true positives" if the same bacterium was isolated in initial and confirmatory cultures; false positive "machine failures" if no bacteria were isolated in initial cultures; false positive "contamination during sampling" if bacteria were present in the initial culture but not in confirmatory testing; and, "indeterminate" if no platelets were available to confirm initial results. "False negatives" were those units captured during QC testing that were missed in early screening.

Results: Routine screening revealed similar rates of true positive and contamination false positive results between buffy coat and apheresis platelet concentrates ($P > 0.05$). In contrast, false positive machine failures and indeterminate results were higher in apheresis units than buffy coat pools ($P < 0.0001$). During QC testing, the rates of true positives (i.e., false negative results), contamination false positives, and indeterminates were similar between both platelet types ($P > 0.05$). QC false positive machine failures were higher in apheresis units than buffy coat pools ($P = 0.0004$). Seventy-five bacteria were isolated during early screening including Gram-positive and Gram-negative organisms while all 15 QC isolates were Gram-positive bacteria. From 2010 to 2016, six septic transfusion reactions (including one fatality) were reported to Canadian Blood Services for approximate rates of 1/100,000 and 1/500,000 septic reactions and fatalities, respectively. Bacteria involved in these reactions included coagulase negative staphylococci (3) and *Staphylococcus aureus* (3).

Summary / Conclusions: Screening of platelet concentrates for bacterial contamination at Canadian Blood Services has reduced septic transfusion reactions. However, detection of false negative screen cultures and reports of septic transfusion events reveals a residual safety risk that merits further intervention.

4A-S18-05

INTRODUCTION OF A GERMAN UNIFORM DONOR QUESTIONNAIRE INTO BLOOD DONOR SCREENING

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Background: Before blood donors are qualified for blood donations they have to answer a donor questionnaire. In 2016 a new national uniform donor questionnaire was introduced in our blood donor service. The new donor questionnaire was improved regarding intensive questions to potential sexual transmitted infections due to new sexual partners within the last four months.

Aims: All donors who answered one question regarding sexual risk behavior (new sexual partner, sexual partner with AIDS or hepatitis, paid sexual partner, bi-sexual partner or homo-sexual partner) with "YES" were deferred for blood donation for 4 month. In a case control study we analyzed the incidence rate for HBV, HCV, and HIV-1 for first time donors and multiple time donors asked with the old donor questionnaire in 2015 and with the new donor questionnaire in 2016.

Methods: All donors were screened by serology methods with ABBOTT PRISM for HBsAG, Anti-HCV, HIV combo and Anti-HBc and in parallel by NAT with the German Red Cross PCR system and by Roche Cobas MPX and DPX.

Results: Between June and November 2015 (control period) and June and November 2016 (case period, new donor questionnaire) 23,124; 21,534; 289,033 and 282,617 first time donors and multiple time donors were analyzed in the control study group and the case study group, respectively. Table 1 shows the incidence data for HIV-1, HBV and HCV.

Summary / Conclusions: Although all donors with potential sexual infectious risks were deferred with the new donor questionnaire (no blood collection for deferred donors) the incidence data did not show a clear picture. For first time donors the incidence rate for HCV was higher in 2016 compared to 2015 whereas the incidence rate for HBV was vice versa. The picture was complete the opposite for multiple time donors. Therefore a new study is planned in 2017 with a sample collection for all deferred blood donors with potential sexual infectious risks. These new data might answer the question about the relevance of donor questionnaire in the presence of blood donor screening by NAT and serology methods.

4A-S18-06

REDUCING RISK OF ZIKA VIRUS TRANSMISSION BY WHOLE BLOOD MIRASOL TREATMENT OR CELL WASHING

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Background: Intrauterine blood transfusion may be life-saving in preterm Rh isoimmunized pregnancies in the developing countries. Given the potential for ZIKA virus transmission through blood transfusion and the absence of laboratory screening for the virus in developing countries, a practical alternative approach to mitigate possible transmission by whole blood is pathogen reduction technology (PRT). Among

the existing PRTs, Mirasol (Terumo BCT) is the most advanced platform for whole blood and is effective for virus reduction for a broad range of pathogens.
Aims: To investigate the effectiveness of the Mirasol PRT system at inactivating Zika virus in whole blood.
Methods: Zika virus strains Uganda (ATCC), Puerto Rico (CDC, Atlanta, US) and Thailand (National Microbiology Laboratory, Canada) were used to inoculate whole blood units that then underwent treatment with riboflavin and UV light according to the manufacturer's instructions. Samples were removed before and after treatment and titrated on 96-well flat bottom tissue culture plates seeded with Vero cells. Virus in plasma, red blood cells (RBCs) and platelets (PLTs) was quantified as tissue culture infectious dose 50%/ml endpoint titer (log TCID50/ml) calculated by the method of Spearmen-Carber.
Results: Plasma from whole blood spiked with Zika-Uganda strain had a pre-Mirasol treatment titer of 7.45 logs TCID50/ml and a post-Mirasol treatment of 5.89 logs TCID50/ml or a reduction of 1.56 logs TCID50/ml. Packed RBCs (approximately $4 \times 10e9$/ml) prepared from both pre-and post-Mirasol treated whole blood were washed 3x with saline to remove residual plasma, lysed with 1.5 ml of H2O, freeze-thawed 3x and further diluted 1:10 to avoid cytotoxicity when infecting Vero cells. While the first two washes contained residual Zika virus, the third wash and the packed RBCs were not infectious; given the dilution factor the potential residual infectivity of the washed RBCs was less than $2.5 \times 10e1$/ml. PLT pellets prepared from 10 ml pre-and post Mirasol treated spiked whole blood contained around $300 \times 10e6$ washed platelets contaminated with other cells (~60x10e6 RBCs and 200,000 WBCs); these were resuspended in cell media, freeze-thawed 3x and used for infecting fresh Vero cells. Both PLT preparations were not infectious. These results were replicated after storage of spiked whole blood for 22 days; Zika virus was quite stable at +4C with only 0.7 logs TCID50 reduction compared to day 0. However, neither RBCs nor PLTs were infectious after 3x washing. This observation is important and suggests that unlike its close "cousin", Dengue virus which can penetrate both RBCs and PLTs and render them permissive for viral replication, Zika virus binding to these cells is reversible by simple washing. The same study design is currently being applied to the Asian and South-American Zika strains.
Summary / Conclusions: The inactivation of Zika virus in whole blood by the Mirasol PRT system is modest. RBC washing is carried out routinely in approximately 1% of donated RBCs and platelets to remove plasma proteins for prevention of allergic reactions. Our results indicate that the same approach could be used for mitigation of Zika infection especially in developing countries where NAT is unavailable.

Clinical: Back to the future – is there a role for whole blood transfusions

4A-S19-01
WHOLE BLOOD AS PRESURGICAL HEMOSTATIC RESUSCITATION FLUID IN PATIENTS WITH TRAUMATIC HEMORRHAGIC SHOCK

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Background: Hemorrhage is the number one preventable cause of death due to trauma both in civilian and military settings. Trauma is the leading cause of death in patients between ages 1 to 46 years and the most common cause of life years lost before the age of 76. Data from recent military conflicts shows that 90% of trauma-related deaths occur prior to surgical intervention. Both military and civilian data estimate that between 20–25% of trauma deaths are preventable, and that 65–90% of these deaths are due to traumatic hemorrhagic shock. The essential elements of pre-hospital care for bleeding trauma victims are hemorrhage control and resuscitation. Pre-hospital resuscitation must address oxygen delivery and hemostasis. A strategy termed Remote Damage Control Resuscitation (RDCR) uses blood products and hemostatic adjuncts to deliver critical physiologic support. By contrast current European guidelines suggest crystalloids and hypotensive resuscitation in the pre-hospital setting. The use of crystalloids for patients with traumatic hemorrhagic shock is debated as they may cause dilution coagulopathy and do not directly improve oxygen delivery or hemostasis.
Aims: Explore the benefits and risks associated with early treatment (pre-surgical) of traumatic hemorrhagic shock with blood products instead of clear fluids. Examine the literature and physiologic basis for current guidelines and discuss alternative practices such as the use of Group O whole blood as the fluid of choice for traumatic hemorrhagic shock resuscitation.
Methods: Clinical studies and studies with a focus on the physiology of traumatic hemorrhagic shock will be reviewed. On-going pre-hospital whole blood programs in the US, cruise liner industry, and Europe will be described.
Results: Recent studies support the use of whole blood as a feasible option for traumatic hemorrhagic shock resuscitation and as a practical way of giving 1:1:1 in the pre-hospital setting. Whole blood supports both oxygen delivery and hemostasis in one product, thus also reducing volume of additive solutions. Emergency settings are known to increase the potential for human errors and evidence indicates that group O whole blood with low anti A/B titer is the safest policy and may be more efficacious and safe than using individual blood components for resuscitation of traumatic hemorrhagic shock. The one bag solution also reduces the logistic burden of far forward blood component availability and facilitates transfusion. RDCR with low titer Group O whole blood is being reintroduced in both military and civilian emergency medicine systems.
Summary/Conclusion: RDCR is the pre-hospital application of established DCR therapeutic approaches utilized in hospital for trauma victims. The awareness of the fact that better care for trauma victims includes early transfusion support should encourage the blood banking community to increase its efforts in making blood products available at the "front line." There is an urgent need to focus on efforts to reduce the number of preventable deaths due to traumatic hemorrhagic shock and to revise current prehospital guidelines that insufficiently address oxygen delivery and hemostasis.

4A-S19-02
COLD STORED VS ROOM TEMPERATURE STORED PLATELETS

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Background: Recognition of the success of whole blood (WB) transfusion in military operational settings has engaged a debate on reintroduction of cold-stored WB in treatment of critical bleeding in civilian health care. The hemostatic function of the platelets contained in cold-stored WB has been questioned.
Aim: This presentation provides an overview of the history of platelet cold storage, discussing the hemostatic properties, clinical effects, and risk of complications associated with cold stored platelet transfusion.
Methods: In vitro studies of the effects of platelet cold storage, whether in WB or platelet concentrates together with in vivo studies on the effect of transfusing these platelet-containing products will be reviewed and discussed.
Results: Platelets were routinely stored cold (4C) until the early 1980's, but practice switched to room temperature (RT) storage due to the longer circulation of RT platelets, thought to be optimal for prophylactic transfusion. Clinical experience, in vitro, and in vivo data, however, suggested hemostatic superiority of 4C platelets in treatment of bleeding. Previous in vitro studies show reduced risk of bacterial contamination and equal or superior hemostatic qualities in 4C platelet concentrates when evaluated by metabolic measures, aggregation response, and other tests of coagulation. With the exception of an ongoing study, randomized clinical trials comparing clinical outcomes of transfusion with 4C or RT platelet concentrates have not been performed since the 1970's. Previous clinical studies in thrombocytopenic patients indicate improved platelet aggregation and reduced bleeding after transfusion with 4C platelet concentrates. Recent in vitro investigations of platelet function in 4C WB during storage are typically performed by use of viscoelastic tests (TEG/ROTEM) or Multiple Electrode Aggregometry. Platelet function shows a decline during storage for up to 21 days by both measures, but platelets in 4C WB show superiority compared to RT WB over the storage period when evaluated by the thromboelastography measure maximal amplitude, MA. Recent clinical studies of WB focus on the feasibility of WB compared to component based therapy in treatment of major bleeding,

but studies comparing platelet effects in 4C vs RT stored whole blood are scarce. A randomized controlled trial in pediatric patients undergoing cardiac surgery showed reduced blood loss and improved platelet function by aggregometry after transfusion with 24–48 h stored 4C WB when compared to component based therapy with RT platelets. Recent animal studies also suggest efficacy and safety of 4C WB. With the exception of an ongoing trial, studies comparing complication risks of 4C platelets with RT platelets have not been identified.
Summary/conclusions: Previous in vitro, in vivo and clinical studies indicate that cold stored platelets may be beneficial in treatment of critical bleeding. Cold storage of platelets may enable extended storage time, facilitate improved availability of prehospital transfusion, and allow shipment of platelet containing blood products to remote, austere environments world-wide. Clinical studies should therefore be performed to investigate the feasibility, safety and clinical efficacy of cold stored platelet concentrates and WB transfusion.

4A-S19-03

IMPROVING COMMUNICATION PATHWAYS AND BLOOD COMPONENT USAGE IN MAJOR OBSTETRIC HAEMORRHAGE

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Background: Major obstetric haemorrhage(MOH) is a leading cause of early maternal death worldwide and a major cause of morbidity in the developed world. To improve patient outcomes, existing guidelines recommend development of local protocols that clarify communication pathways between key personnel and ensure the timely delivery of blood components. All MOH cases should be reviewed in multidisciplinary team (MDT) meetings and protocols should be audited regularly.
The MOH protocol in Leeds Teaching Hospitals Trust (LTHT) was amended in 2015, with particular focus on improving the speed of delivery of blood components to labour wards. In January 2016, changes were made to the contents of the 'haemorrhage pack', moving from a 2:1 RBC:FFP pack to a 1:1 pack which also contains platelets. The updated policy recommends using pre-thawed FFP as well as the use of 1 gram of Fibrinogen.
Aims: To document improvements in clinical practice since changes were made to the MOH pack and the communication pathways.
Methods: In Leeds Teaching Hospitals Trust (LTHT) the compliance with MOH protocol is audited quarterly against the following standards:

- MOH protocol trigger.
- Presence of designated communicator in the clinical area and in the transfusion laboratory.
- Timely delivery of MOH pack.
- De-escalation of protocol.

Results: 6 audits were completed between 7/2015 and 1/2017. During the study period the MOH protocol was triggered in 64 cases of MOH, approximately 11 cases quarterly.
The 1st audit (July - October 2015) showed the MOH protocol was triggered in 30% of the cases by a designated MOH bleep and the remaining cases by a telephone call; a designated communicator was present in the clinical area in 0% and in the transfusion laboratory in 100% of cases, and the protocol was de-escalated in 11% of cases.
The 6th audit (October - December 2016) showed the MOH protocol was triggered in 66% of the cases by a designated MOH bleep, a designated communicator was present in the clinical area in 30% and in the transfusion laboratory in 100% of cases, and the protocol was de-escalated in 11% of cases.
After implementation of the new MOH protocol appropriate usage of components increased; for platelets from 56% to 74%, FFP from 71% to 73%, cryoprecipitate from 60% to 100% while that of RBC remained unchanged (56%).
The average time of MOH pack release from the transfusion laboratory to the labour wards was reduced from 31 min to 5.6 min over the study period of 18 months
Summary / Conclusions: Regular audits of the MOH protocol have improved the transfusion management of MOH patients in LTHT.
MDT review of the audit reports resulted in amendment of MOH protocol and significant reduction in the time required for release of MOH packs and improvement of the use of blood components, particularly platelets and FFP, The communication between transfusion laboratory and the clinical area has improved since introduction of the MOH bleep. The audits have identified areas for improvement mainly de-escalation of the protocol and the allocation of a designated clinical communicator.

4A-S19-04

ARE SAMPLING PRACTICES LEADING TO TOO MUCH BLOOD IN THE LAB AND LESS IN THE PATIENT?

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Background: Patient blood management (PBM) focuses on conserving a patient's own blood as a means to improving patient outcomes, thus reducing the need for transfusion and its associated risks. Strategies to reduce iatrogenic blood loss are an important blood conservation method, however; policy supporting this appears lacking. Reduction of iatrogenic blood loss requires strategic planning, communication and implementation with relevant stakeholders such as medical staff, laboratory scientists and nursing staff.
Aims: To determine the prevalence of local guidelines addressing minimisation of volume and frequency of blood sample collection to prevent iatrogenic anaemia in health services in four Australian jurisdictions. To describe the practice of these health services to reduce blood loss due to blood sampling and to identify any barriers to implementing such strategies.
Methods: Public and private health services in rural and metropolitan areas (n = 149) were invited to respond to an online audit related to local policies that document strategies to support practices to reduce iatrogenic blood loss. Strategies included in the audit were those recommended in recent peer-reviewed literature; namely, small volume phlebotomy tubes, closed system sampling, frequent evaluation of routine blood sampling orders, bundled scheduling of blood sampling, point of care testing, non-invasive monitoring and charting of cumulative daily phlebotomy loss.
Results: Seventy-eight health services completed the online audit (response rate 52%). Only six health services reported having a policy to minimise the volume and frequency of blood sample collection to reduce iatrogenic anaemia. However, 85% (66/78) reported that minimal blood sampling occurred in their health service to some degree; most commonly in paediatrics and intensive care units. Strategies most frequently reported were point of care testing (n = 45), small volume phlebotomy tubes (n = 37), frequent evaluation of routine sampling orders (n = 28) and closed system sampling (n = 19). However, these health services are reporting that these strategies are predominantly occurring for purposes other than to minimise iatrogenic anaemia, most frequently for convenience, time efficiencies or improved patient comfort. Only two health services reported charting of cumulative daily phlebotomy loss specifically in neonatal special care units. In addition, some health services reported practices that may promote unnecessary blood tests increasing the risk of iatrogenic anaemia, such as ordering specified test sets (n = 30) and routine blood sampling orders (n = 19), without adequately evaluating the clinical condition of the individual patient. The most frequent (n = 38) explanation for a lack of policy and/or practice of minimal sampling was due to "practice has not been considered'.
Summary / Conclusions: Currently most health services do not have policies supporting minimal blood sampling as a PBM approach. It appears this has not been considered although minimal blood sampling strategies are being used widely for reasons other than prevention of iatrogenic anaemia. Audit recommendations include: increasing awareness of iatrogenic anaemia and potential benefits of strategies to reduce this, development of an audit tool to determine the cumulative daily phlebotomy loss per patient to highlight the need for practice change.

Young Investigator Session

4A-S20-01

THE INS AND OUTS OF SMIM1 AND ITS RELATIONSHIP TO THE EXPRESSION OF VEL BLOOD GROUP ANTIGEN

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Background: Vel blood group expression is dependent on Small Integral Membrane Protein 1 (SMIM1), a recently discovered erythroid protein. SMIM1 consists of 78 amino acids (aa) and shows only limited homology to other human proteins but is evolutionarily conserved, indicating its importance. The protein has a predicted transmembrane domain but the direction of insertion into the red blood cell (RBC) membrane is not yet clarified. The aa sequence contains reactive residues such as cysteines with the potential for disulphide bridge formation and a GXXXG motif

known to induce dimerization in glycophorins. Many proteins form multimers to become functional. Thus, due to SMIM1's capability to dimerize and its association with mean corpuscular haemoglobin concentration (MCHC), we hypothesized that the above-mentioned motifs are of importance.
Aims: The aim of this study was to investigate two features of SMIM1; GXXXG and the cysteine residues. Through mutagenesis studies, we investigated their role in Vel antigen expression and dimerization.
Methods: Glycine in the GXXXG motif (aa 67-71) of the predicted transmembrane portion was substituted for leucine. Cysteine at positions 35, 43 and 77 was substituted for alanine. The SMIM1 constructs (incl. mock and wild-type) were transiently expressed in K562 cells. Vel surface expression was analysed using flow cytometry (FACS) with human polyclonal anti-Vel. The total protein content and dimerization was analysed by Western blot with a rabbit polyclonal antibody produced against the N-terminus (aa 1-15) of SMIM1. α-chymotrypsin treatment was performed as described [Storry et al. Nat Genet, 2013].
Results: Mutation of either or both of the glycines in the GXXXG motif caused significant reduction of Vel surface expression compared to SMIM1 wild-type, although the SMIM1 protein content remained unchanged as evaluated by Western blot. Substitution of p.Cys77Ala also significantly reduced Vel antigen expression, while no effect was observed by p.Cys35Ala or p.Cys43Ala. Again, the SMIM1 content appeared unaffected by the substitutions. The capacity to form dimers remained intact in all mutants. To verify the location of the N-terminus, α-chymotrypsin treatment of intact RBCs was performed. Absence of SMIM1-characteristic bands on Western blot of RBC membranes indicated that the N-terminal target for the rabbit antibody used was destroyed.
Summary / Conclusions: Vel surface expression is severely affected by substitutions of glycine at positions 67 and 71, or of cysteine at position 77 in SMIM1. Disruption of a transmembrane localisation signal may explain the importance of glycine substitutions. The importance of Cys77 for Vel antigenicity could indicate an extracellular location of the C-terminus. This appears to be consistent with conclusions from the study by Arnaud et al. [FEBS Lett., 2015]. However, this is somewhat contradicted by our results from α-chymotrypsin treatment of intact RBCs. If the C-terminus is indeed extracellular and SMIM1 a type II protein, the N-terminus should be insensitive to such treatment.
In summary, we conclude that the targeted GXXXG motif and Cys77 are important for the correct, extracellular exposure of the Vel antigen. It remains unclear if SMIM1 is a type I or II transmembrane protein. This question deserves further investigation to facilitate studies on functional aspects of this enigmatic protein.

4A-S20-02

PURIFIED PROTHROMBIN TRANSFUSED AT THE TIME OF INJURY REDUCES BLEEDING IN BOTH NORMAL AND COAGULOPATHIC MICE

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Background: Administering purified coagulation factor concentrates is effective in remedying the bleeding tendencies caused by single factor deficiencies in human genetic diseases and in their mouse gene knockout models, for instance in hemophilia A or B. Typically, the missing factor is replaced by infusion of the corresponding factor concentrate (e.g. purified Factor IX (FIX) in hemophilia B). Recently it has been reported that the infusion of a different clotting factor, purified prothrombin, derived either from human plasma or transfected cell cultures, reduces bleeding in murine models of either hemophilia A or B (Hansson KM, Lindblom A, Elg M, Lövgren A. Haemophilia 2016;22:453-61). We hypothesized that supraphysiological doses of prothrombin could also relieve coagulopathy and/or reduce bleeding in either acquired, multi-factor deficiency or in a surgical setting.
Aims: To examine the impact of purified human prothrombin administration on blood loss subsequent to tail transection or liver laceration in either coagulopathic mice with an induced pan-factor deficiency or in normal mice.
Methods: Purified human prothrombin and purified FIX were purchased and purity was confirmed using anti-human factor antibodies and immunoblotting. Coagulopathy was induced following a previously described Blood Exchange-induced Coagulopathy Approach (BECA) in CD1 mice. This approach involves four sequential exchanges of whole blood for washed red cells, reducing all coagulation factors in plasma to 20% of normal without creating shock. BECA mice were transfused with treatment solutions (either 5% Human Albumin Solution (HAS) vehicle or prothrombin or FIX diluted in vehicle) immediately prior to: tail vein transection; or liver transection. Normal mice were also challenged by liver transection. All values are means ± SD, n = 12–15.
Results: Normal mice subjected to surgical liver laceration and treated with 3.6 mg/kg prothrombin lost 130 ± 30 mg of blood compared to vehicle-treated mice (220 ± 80 mg, P = 0.012). In BECA mice, liver laceration was associated with indistinguishable blood losses of 610 ± 80 mg and 610 ± 80 mg in mice treated with vehicle or 10 mg/kg FIX; prothrombin-treated mice loss significantly (P < 0.001) less blood (340 ± 110 mg). BECA mice subjected to tail transection with vehicle treatment lost 270 ± 100 µl of blood, significantly (P < 0.001) and indistinguishably reduced by 12 ml/kg murine plasma transfusion (70 ± 50 µl) or 3.6 mg/kg (100 ± 50 µl) or 10 mg/kg (54 ± 40 µl) prothrombin.
Summary / Conclusions: Infusions of purified human prothrombin, but not factor IX, ameliorate the bleeding diathesis in normal mice with surgical bleeding or coagulopathic mice with either internal or external hemorrhagic injuries. Additional prothrombin can therefore productively drive coagulation in pan-factor coagulopathy. These observations may explain reports of a general anti-hemorrhagic effect of prothrombin-containing plasma protein products such as prothrombin complex concentrates.

4A-S20-03

FUNCTIONAL ANALYSIS OF RHD VARIANTS: A UNIVERSAL TEST TO INVESTIGATE SPLICING ALTERATION

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Background: Splicing is a key step of the post-transcriptional regulation of genes. A modification occurring in the vicinity of splice site may result in an alteration of the phenotype. For a few years we have developed a functional approach to study the molecular consequences of splice site variants based on a plasmid construct, called "minigene vector". This plasmid tool, which has shown its potency to study the functional consequences of splice site variants affecting internal exons of the *RHD* gene involved in the Rh blood group system, was recently upgraded to analyse the effect of variants in exon 1.
Aims: We sought 1) to upgrade the minigene construct to generate a universal tool for the analysis of all potential splice site variations, including those affecting the last exon of a gene; 2) to test a sensitive, fluorescent RT-PCR approach to gain insights into the defect resulting from a variation both at the qualitative and quantitative levels; 3) to validate our model and test the novel minigene construct by studying 13 mutations within the *RHD* gene whose functional impact on splicing is still unknown.
Methods: The original plasmid was modified by site-directed mutagenesis to create three different, single enzyme restriction sites allowing subcloning of short gene regions of interest originating from either 1) the first exon, 2) an "internal" exon or 3) the last exon by a homologous recombination-based approach. Eighteen wild-type and mutant *RHD* gene constructs were produced. After transfection in a eukaryotic cell model, transcript expression was studied by RT-PCR of short fluorescent fragments, cloning and sequencing.
Results: Effect of splice site variants located at internal exon-intron junctions of the *RHD* gene was tested in all "minigene" versions with similar results, suggesting that the novel pMG3.1 universal minigene can be used for minigene splicing assay. No effect on splicing was observed with variants c.146A>G, c.147A>G, c.148 + 5G>C, c.149T>A, c.635G>A and c.1228T>G. Five variants showed a total disruption of the natural donor or acceptor splice site (c.148 + 1G>T, c.148 + 1G>A, c.635-2A>C, c.939 + 1G>A and c.1228-1G>A), while two other variants showed a partial disruption (c.634G>A and c.1154G>A).
Summary / Conclusions: Our universal minigene is relevant to easily assess the functional effect of all variations in the vicinity of splice site, whatever exon may be affected. Moreover alternative transcripts resulting from activation of a cryptic splice site may be easily identified by the fluorescent RT-PCR-based approach. Correlation between functional analysis and phenotype is clear in 6/7 variants (i.e. partial/total disruption of splice site associated to weak, DEL, or D-negative phenotype), but c.1154G>A exhibited unexpected results. Indeed splicing is barely impaired, while a D-negative phenotype has been reported with this variation, suggesting that expression of D antigen is completely impaired by the effect of amino acid substitution (p.Gly385Asp) at the protein level. Additional studies should therefore be considered to understand the effect of the missense mutation on the phenotype.

4A-S20-04

LOW-GRADE INFLAMMATION AMONG HEALTHY PREMENOPAUSAL WOMEN TAKING COMBINED ORAL CONTRACEPTION DEPENDS ON DOSE AND TYPE OF PROGESTIN: RESULTS FROM THE DANISH BLOOD DONOR STUDY (DBDS)

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Background: C-reactive protein (CRP) is a well-established marker of inflammation and several lifestyle factors affect the level of CRP. A slightly elevated CRP level, also known as low-grade inflammation (LGI), is associated with increased risk of several diseases, e.g. cardiovascular disease. Recently, we reported that the use of combined oral contraception (OC) was the strongest predictor of LGI among premenopausal women and indeed stronger than high body mass index (BMI) and abdominal obesity. The mechanism behind this effect of OC is not known, but the effect is substantial: LGI was present in 29.9% of OC users and 7.1% of non-users among premenopausal female blood donors.
Aims: The aim of this study was to examine the impact of distinct groups of OC on the risk of LGI in a large cohort of blood donors. The hypothesis was that a higher content of estrogen and progestin was positively associated with the risk of LGI.
Methods: Plasma CRP levels in 6,989 women from The Danish Blood Donor Study were measured by a commercial assay. All participants completed a standard questionnaire on lifestyle factors, and further stated their use of contraception, childbirth and menopausal status. Type of OC was identified by ATC codes in the Danish National Prescription Registry. Indications for OC use include gynecological disorders such as irregular menstrual bleeding, endometriosis or PCOS. As these conditions might independently cause LGI, women with these diagnosis (N = 41) were excluded from the study. Association between LGI (defined as CRP level >3 mg/l and <10 mg/l) and OC groups (classified as 2^{nd}, 3^{rd} and 4^{th} generation decided by the type of progestin) was explored by multivariable logistic regression analysis. Adjustments for BMI, age and physical activity in leisure was performed. Results were presented as odds ratios (OR) with 95% confidence intervals (CI).
Results: A total of 1,823 (26.5%) female participants were users of OC. Of these, there were 253 (13.7%), 1,290 (69.6%) and 310 (16.7%) users of 2^{nd}, 3^{rd} and 4^{th} generation of OC, respectively. LGI was found more frequent among users of OC with 2^{nd} generation (35.2%) than 3^{rd} generation (31.0%) and 4^{th} generation (24.2%). Among premenopausal women using OC, the use of 4^{th} generation predicted a lower risk of LGI compared to 2^{nd} generation as reference (OR = 0.68, CI: 0.46;0.99, P = 0.044). No statistical significant difference between 2^{nd} and 3^{rd} (OR = 0.94, CI: 0.70-1.27, P = 0.71) or 3^{rd} and 4^{th} generation was observed.
Summary / Conclusions: The use of 4^{th} generation OC was associated with a significantly lower risk of LGI compared to 2^{nd} generation OC. The finding indicates that the CRP increase found in OC users depend on type and content of progestin. The finding underscores that blood donor cohorts are feasible for the study of health properties in healthy persons. The underlying mechanism of OC and potential impact of OC linked LGI on risk of disease remain to be investigated.

4A-S20-05

USING A DATA DRIVEN INVENTORY REPLENISHMENT MODEL TO REDUCE BULLWHIP IN SCOTLAND'S BLOOD SUPPLY CHAIN

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Background: Bullwhip is a phenomenon where the end customer demand signal is amplified as it moves up the supply chain. It causes inefficiencies such as surplus inventory which can lead to time expiry waste, disruption to production schedules and increased transport costs. Analysis of Red Blood Cell (RBC) transaction data throughout Scotland's blood supply chain has found evidence of bullwhip and identified hospital blood bank ordering practice (order inflation and batching) as the biggest contributor to demand amplification. This paper presents the results of a hospital case study that implemented a data driven inventory replenishment model in an attempt to mitigate the bullwhip effect.
Aims: Determine the impact of a data driven inventory replenishment model on blood bank ordering volatility and the bullwhip effect for ANEG, APOS, BPOS, ONEG, OPOS RBC components.
Methods: An Excel-based dynamic inventory replenishment model was installed in the blood bank at a large acute hospital in West Scotland. The model was driven by daily demand data and used a Simple Exponential Smoothing forecast to calculate Target Stock Levels required to generate recommended order quantities. Between 22/11/2016-16/01/2017, staff followed the recommended ordering advice generated each day by the model for 5 RBC components.
Order volatility and bullwhip for the 5 components during the pilot were compared with a baseline period (04/04/2016-29/05/2016). Daily order quantities and transfusion data from the NHS NSS Account for Blood datamart for the two periods were statistically analysed to determine changes. Order volatility was calculated using the Coefficient of Variation (CoV) of daily orders. Bullwhip was measured using the Amplification Ratio (AR = ratio of CoVs of daily orders and daily transfusions).
Results: Order volatility decreased for all components with the exception of ANEG RBCs. APOS ordering volatility decreased by 3%, whilst BPOS, ONEG and OPOS saw reductions of 19%, 7% and 15% respectively. The reduced volatility was a result of less variability in order size and a smoothing of the order profile.
Bullwhip analysis revealed a 64% reduction in AR for ONEG; APOS (13%), BPOS (38%) and OPOS (37%). However, the AR for ANEG increased by 18% due to a non-routine increased volatility in orders in response to several major haemorrhage events.
Further analysis into average component age at transfusion revealed a reduction in age of between 0.7 days (APOS) to 7.2 days (BPOS). Use of costly supplementary taxi deliveries of RBC components decreased by 50% during the pilot period.
Summary / Conclusions: The inventory replenishment model reduced order volatility for 4 components, and demand amplification (bullwhip) between the hospital blood bank and blood supplier echelon. The results demonstrate the benefits of using a data driven model for component ordering to remove volatility introduced by current ordering practice. It helped to achieve cost savings through more appropriate use of deliveries, as well as enabled the provision of fresher blood to patients.
If all blood banks adopted measures to reduce order volatility, then the cumulative effect would smooth the aggregate demand signal thus reducing pressure on blood processing and collection activities, and improve the match between supply and demand.

4A-S20-06

PACKED RED BLOOD CELL STORAGE DURATION AND MORTALITY: PRELIMINARY RESULTS FROM POOLED PATIENT ANALYSIS

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Background: During *in vitro* storage, units of packed red blood cell (PRBCs) accumulate cellular and biochemical changes collectively called the PRBC storage lesion. Data regarding the clinical significance of the PRBC storage lesion have been equivocal with the majority of "positive" findings from observational studies – suggesting that the effect may be limited to a select population. Furthermore, observational studies tend to ask "Are old PRBCs associated with harm?" whereas randomised controlled studies tend to ask "Are young PRBCs associated with benefit?". Recent meta-analyses combining data from observational studies used multiple adjustments to create two PRBC storage duration groups due to differences in aggregate PRBC storage duration reporting (e.g. mean vs. dichotomous groups <21 days, >21 days). This problem can be abrogated by using patient level data.
Aims: This study aimed to complete a pooled patient analysis of 14 observational studies to determine if there was an association between increasing mean storage duration of PRBCs and post-transfusion in-hospital mortality.
Methods: Observational studies reporting PRBC storage duration and clinical outcomes were identified from PubMed and EMBASE. Corresponding investigators were contacted to request the underlying patient-level dataset. Institutional approvals were sought prior to receiving each dataset. First, the association of mortality with

mean storage time was estimated and reported as an odds ratio (OR) for each study with age, gender, and PRBC volume as covariates. Effect estimates were then pooled using a random effects model. STATA (version 14.0) was utilised for all analyses.
Results: The computerised search retrieved 3,285 studies with 58 publications satisfying the selection criteria. Of these, 14 datasets consisting of 14,867 patients and 58,272 transfusions were made available for this meta-analysis. There was no association between mean storage duration of PRBC transfused and post-transfusion in-hospital mortality (odds ratio: 0.989, 95% CI 0.976-1.003). The funnel plot was symmetric suggesting that publication bias was not a concern.
Summary / Conclusions: Pooled patient analysis of 14 observational studies did not identify any association between mean PRBC storage duration and post-transfusion in-hospital mortality. Further analyses are underway to investigate the effects of PRBC storage duration on infection and hospital length-of-stay.
Acknowledgements: Australian governments fund the Australian Red Cross Blood Service for the provision of blood, blood products and services to the Australian community. This project was partially funded by The Prince Charles Hospital Foundation.

Plenary session: New developments

PL2-01

A NEW APPROACH TO TREATING PATIENTS WITH BETA THALASSAEMIA

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The thalassaemias, together with sickle cell anaemia and its variants, are the world's most common forms of inherited anaemia, and in economically undeveloped countries, they still account for tens of thousands of premature deaths every year. In developed countries, treatment of thalassaemia is also still far from ideal, requiring lifelong transfusion or allogeneic bone marrow transplantation. Clinical and molecular genetic studies over the course of the last 50 years have demonstrated how co-inheritance of modifier genes, which alter the balance of α-like and β-like globin gene expression, may transform severe, transfusion-dependent thalassaemia into mild forms of anaemia. Most attention has been paid to pathways that increase γ-globin expression, and hence the production of foetal haemoglobin. In this presentation we will review the evidence that reduction of α-globin expression may provide an equally plausible approach to ameliorating clinically severe forms of β-thalassaemia, and in particular, the very common subgroup of patients with haemoglobin E β-thalassaemia that makes up approximately half of all patients born each year with severe β-thalassaemia. Genome engineering of haematopoietic stem cells may provide a new approach to treating patients with β-thalassaemia.

PL2-02

HEPATITIS E

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For several reasons hepatitis E virus genotype 3 (HEV-3) stands apart from the classical viruses that threaten blood safety. It is less pathogenic, and via transfusion it is less infectious than HBV, HCV and HIV. In some Western countries anti-HEV seroprevalence studies suggest that HEV-3 returned after having been away for decades. The main natural transmission route probably is consumption of undercooked pig meat products. HEV-3 seems harmless for most healthy persons, including pregnant women and neonates. In fact, a massive silent epidemic is going on in some regions and countries, with a silent incidence of 1%/year in the Netherlands. HEV-3 can cause disease: especially older men are at-risk for symptomatic, mild and self-limiting acute hepatitis. Sporadic cases of severe neuralgic amyotrophy can be attributed to HEV-3 infection. HEV-3 specifically affects immunosuppressed persons such as stem cell transplant patients and solid organ transplant patients. In these patients HEV-3 infection often causes chronic hepatitis with mild and fluctuating ALT elevation, which can rapidly lead to fibrosis of the liver. In these patients IgG and IgM anti-HEV may remain negative for months or years, hence the screening for- and diagnosis of chronic hepatitis E must be based on detection of HEV RNA by PCR. Careful reduction of immunosuppression cures chronic hepatitis E in some patients. An oral course of ribavirin clears chronic hepatitis E in most affected patients. In immunosuppressed patients relapses and re-infections occur. During ribavirin treatment in some patients HEV-3 appears to develop ribavirin-resistance via specific mutations.
HEV-3 consequences for blood banking.
For blood banking HEV-3 poses a dilemma. To protect vulnerable patients against blood-borne infection with HEV-3, Irish, British and Dutch health officials decided to introduce the screening of blood donors for HEV by PCR. At the same time no action is taken to remove HEV-3 from the food chain. We estimate that food-borne transmission of HEV-3 is roughly 700 times more common than blood-borne transmission. For the Netherlands we estimate that per year 133.000 food-borne and 187 blood-borne HEV-3 infections occur. Of course hematological patients often are exposed to high numbers of blood products, for them the risk of blood-borne HEV-3 infection is relatively high. The screening of Dutch donors for HEV-3 seems reasonably cost-effective: the estimated cost per chronic hepatitis E case averted would be approximately Euro 310.000, applying universal screening in pools of 24.
On the governmental level (including the EU), the lack of concern regarding food-borne HEV-3 transmission is striking, so far economic interests seem to prevail. Some HEV-3 related studies are undertaken, but they probably will confirm what we already know. A more pro-active course would be desirable. For example, it should be investigated which production processes in the food industry do not inactivate viruses like HEV. Since years we know that a significant part of EU piglets acquire HEV-3 infection, resulting in approximately one in ten Dutch pig livers being HEV-3 positive at the time of slaughter. Maybe the solution is simple: if all piglets were to be infected with HEV-3 deliberately at young age, the infection would be resolved at the time of slaughter.

PL2-03

INVESTIGATIONAL BABESIA MICROTI BLOOD DONATION SCREENING IN ENDEMIC AREAS IN THE UNITED STATES; CURRENT AND FUTURE TRENDS

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Introduction: Babesia microti is a transfusion-transmissible intraerythrocytic parasite; incidence is increasing and no FDA-licensed blood donation screening assay is available. We conducted blood donation screening using investigational assays to detect DNA and antibodies to Babesia microti.
Methods: From June 2012 through March 31, 2017, we preformed arrayed fluorescence immunoassays (AFIAs) for B. microti antibodies and real-time polymerase chain reaction (PCR) assays for B. microti DNA on blood donation samples obtained in Connecticut, Massachusetts, Minnesota, and Wisconsin. We determined parasite loads with the use of quantitative PCR testing and assessed infectivity by means of the inoculation of hamsters and the subsequent examination for parasitemia. Data from this study and a repository study were combined to assess DNA and antibody duration in donors. We compared the proportions of screened versus unscreened donations that were implicated in cases of transfusion-transmitted babesiosis (TTB).
Results: Of 312,473 donations screened, 964 (0.3%) tested reactive of which 144 (14.9%) were PCR-reactive on screening PCR or enhanced (e)PCR; 16 donations were antibody-negative (i.e., 1 antibody-negative donation per 19,529 screened donations), representing 11.1% of all PCR-reactive donations. PCR-reactive donations were identified in every month of the year; PCR-reactive/antibody-negative donations were identified primarily from June through September, although one was identified in February. The median parasite load in PCR confirmed-positive donations was 358 parasites/mL (interquartile range, 27–2,744; overall range 5–2.99 million). Approximately one-third of the red cell samples from PCR confirmed-positive or high-titer AFIA confirmed-positive donations infected hamsters. Follow-up of infected donors showed DNA clearance in 88.1% of the donors and antibody seroreversion in 8.3% after one year. In the highly endemic states of Connecticut and Massachusetts, no reported cases of transfusion-transmitted babesiosis were associated with screened donations (i.e., 0 cases per 312,473 screened donations), as compared with 23 cases per 1,254,819 unscreened donations (i.e., 1 case per 66,296 unscreened donations) (odds ratio, 11.7, 95% confidence interval, 0.7 to 192.7; $P = 0.01$).

Overall, 51 cases of transfusion-transmitted babesiosis were reported during the study period and linked to blood from infected donors, including 20 donors whose samples tested PCR positive one to seven months after the implicated donation was collected.
Conclusions: These data suggest that blood donation screening in highly endemic areas removes infectious products from the blood supply and prevents cases of TTB. Followed donors appear to retain PCR positivity over several months but retain antibody reactivity for one year or longer. Since infectivity is related to the presence of parasite nucleic acids, future trends in screening include ultrasensitive automated nucleic acid tests capable of detecting multiple Babesia spp. In addition, pathogen inactivation has been shown to be effective in reducing at least 6 logs of B. microti red cell infectivity.

Parallel sessions

Blood products: Metabolomics in blood banking and TM

4B-S21-01
METABOLOMIC ANALYSIS OF BLOOD STORAGE
O Sigurjonsson

No Abstract available

4B-S21-02
BIOMARKERS DEFINING THE METABOLIC AGE OF RED BLOOD CELLS DURING COLD STORAGE
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The continued development of omics technologies has allowed for the generation of massive amounts of Big Data that are brimming with information. However, accessing this information is made difficult by the disparate types of data collected, a lack of well-defined analytical and computational workflows, and the segregation of data. One of the major challenges faced by the transfusion medicine community is therefore to analyze these data sets and start to access the information hidden within. Over the past several years, systems biology analysis has revealed distinct metabolic phenotypes during the red cell storage process. Biomarkers have been identified that reliably define this process under multiple storage condition and media perturbations. Further, these biomarkers can be used to quantitatively predict the concentration profiles of other metabolites in the network. By using systems biology to view red cell physiology through an integrative lens, we can start to access the full potential of the Big Data being generated.

4B-S21-03
ANTI-CD47 INDUCES DIFFERENT DEGREES OF PHOSPHATIDYLSERINE EXPOSURE IN RED BLOOD CELLS
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Background: CD47 is a transmembrane receptor with 5 membrane-spanning segments and also is known as Integrin-associated protein (IAP). CD47 is 47–52 kDa on SDS–PAGE and it is a member of the immunoglobulin (Ig) superfamily of membrane proteins. It has a glycosylated N-terminal extracellular immunoglobulin variable domain (IgV). The IgSF domain within CD47 drives its interactions with its receptors and ligands, which include $\alpha v\beta 3$, $\alpha 2\beta 1$ integrins, thrombospondin-1 (TSP-1) and signal-regulatory protein alpha (SIRPα) which behaves as an inhibitory "do not eat me signal" that leads to recognition of self.
Eryptosis is known as the mechanism of RBC death. It shares some properties with apoptosis, which takes place in all other cell types, like phosphatidylserine exposure, membrane blebbing and cell shrinkage.
CD47 monoclonal antibodies (mAbs) are thought to bind to the external N-terminal of CD47 and have been shown to activate the eryptotic pathway by switching phosphatidylserine (PS) from the inner leaflet to the outer leaflet of the membrane.
There are seven different CD47 mAbs from International Blood Group Reference Laboratory (IBGRL) BRIC antibodies (32,122,124,125,126,168 and 211) and these have been tested on RBCs from normal blood donors to evaluate their effects on blocking CD47 and inducing eryptosis in RBCs.
Aims: Evaluation of the ability of different CD47 antibodies to block CD47 but not induce eryptosis in RBCs and to investigate their epitope mapping by competitive binding.
Methods: Red cells from ten random blood donors were obtained from NHSBT, Filton, UK. A flow cytometry assay was used to characterize the CD47 mAbs. Briefly, fresh packed red blood cells were washed at least 3 times with HEPES buffer. Cells were counted by using a haemocytometer. $1*10^6$ cells were incubated with 10 mg/ml of each mAb, for an hour at 37°C. Cells were then washed three times with 500 ml Hanks buffer. Supernatants were removed and the cells re-suspended in 100 ml binding buffer. 3 ml of Annexin V-FITC (BD, U.K.) was added to the cells. Samples were transferred into FACS tubes and analysed by FACS ARIA II (BD, U.K). Also, FACSARIA II software was used to analysis flow cytometry results while Graph pad software was used to calculate *t*-test.
Results: Data shows that BRIC 32, and 122 bind to RBC CD47 with minimal PS exposure, while BRIC 125,126,168, and 211 induce eryptosis via PS exposure. Moreover, different mAb combinations were tested to find out if synergistic effects are found or whether competitive binding is demonstrated. Our data suggest that BRIC 32/122 bind to different CD47 epitopes compared to BRIC 124/125/126/168 and 211.
Summary / Conclusions: We have shown that PS exposure induced by RBC binding with various anti-CD47 mAbs is variable, with BRIC 32 and 122 inducing only low levels of PS exposure compared to BRIC 124-126,168 and 211. Studies are underway to investigate the domains to which the anti-CD47 mAbs bind on CD47. We have also shown by RNA-seq experiments that there are five different RBC CD47 isoforms, and experiments are underway to investigate which isoforms are bound by all CD47 mAbs.

4B-S21-04
METABOLOMIC PROFILING HIGHLIGHTS OXIDATIVE DAMAGES IN PLATELET CONCENTRATES TREATED FOR PATHOGEN INACTIVATION AND SHOWS PROTECTIVE ROLE OF URATE
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Background: Research in transfusion medicine is motivated by the desire to deliver the most-compatible and most-efficient blood product to the patient. It is now well accepted that *ex-vivo* platelet concentrates (PCs) experience biochemical alterations and a functional decline known as storage lesions. Photochemical treatments have been introduced to secure PCs against pathogens but are reported to accelerate these lesions.
Aims: The objective of this study was to investigate metabolic changes in stored PCs treated for pathogen inactivation with the INTERCEPT Blood system (Cerus, Concord, USA).
Methods: PCs either untreated (uPCs) or INTERCEPT-treated (iPCs) were sampled along the 7-day storage period. First, metabolites were extracted and analyzed using ultra-high pressure liquid chromatography – high resolution mass spectrometry (UHPLC-HRMS) followed by statistical analysis (unsupervised and supervised methods). Secondly, we investigated the role of urate, a major plasma antioxidant, in the platelet function using flow cytometry-based assays (CD62P, CD42b, Annexin V, DCFH-DA assays).
Results: We observed oxidative damages in stored iPCs compared to uPCs, in particular alteration of the purine and the glutathione metabolism. We showed diminution of antioxidant defenses following INTERCEPT treatment such as the conversion of urate to allantoin, only possible in humans under the action of reactive oxygen species (ROS). Functional assays on platelets in absence or in an excess of urate suggest a protective role of urate in PCs.

Summary / Conclusions: Our results indicate oxidative damages occurring at the metabolic level in stored iPCs. Understanding better the role of antioxidants such as urate in *ex vivo* PCs would definitively provide new insights to ameliorate the storage conditions and preserve the functionality of platelets.

Immunobiology of blood cells: Abstracts selected for oral presentation

4B-S22-01

A ZINC-FINGER DELETION AT ZNF850 DEFINES THE DOMINANT KIDD-NULL RED BLOOD CELL PHENOTYPE (INJK) WITH FAMILIAR MOOD DISORDER

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Background: The Kidd-null blood group phenotype, lacking the urea transporter UT-B1/*SLC14A1* in the erythrocyte membrane, is associated with transfusion risk and urine concentration defect in humans, and depression-like behavior in mice. While the autosomal recessive form is due to biallelic *SLC14A1* mutations, the cause of the dominantly inherited form, referred to an Inhibitor of Jk (InJk) has not been define yet. We have identified five new families originating from the Subbetic area of Southern Spain with this dominantly inherited Kidd-null phenotype.

Aims: The aims of this study were:

- To identify the causative gene for Dominant Kidd-Null red blood cell phenotype (InJk).
- To determine where is a clinical phenotype associated with Dominant Kidd-Null red blood cell phenotype.

Methods: We performed whole-genome linkage analysis, exome sequencing, expression (RT-PCR and Western) analyses in patients' cells, and functional studies in cell lines. Subjects with Kidd-Null phenotype underwent medical and psychological evaluation, and two probands underwent urine concentration test

Results: Linkage analyses revealed a shared haplotype at 19q13 and exome sequencing identified the deletion of a single C2H2 zinc finger-encoding domain of *ZNF850*. An overlapping deletion was identified in a Japanese Kidd-null case. Protein analysis revealed a reduced amount of glycosylated UT-B1 at the erythrocyte membrane with normal *SLC14A1* mRNA levels. Mutant ZNF850 lost the cytoplasmic location in transfected HEK293T cells with respect to ZNF850 wild-type. Most Kidd-null individuals (80.77%) fulfilled criteria for mood and/or anxiety disorder, with increased suicidal risk in the families (7.4 fold, 95% CI: 2.3–16.7, $P = 7.9 \times 10^{-4}$), and reduced ability to concentrate urea in the urine documented in two cases.

Summary / Conclusions: A predicted zinc finger deletion at ZNF850, prevalent in Southern Spain due to a founder mutation, leads to UT-B1 dysfunction and underlies the dominantly inherited Kidd-null red blood phenotype. The phenotype is also associated with subnormal urine concentrating ability, mood and/or anxiety disorder and increased suicidal risk.

4B-S22-02

IDENTIFICATION OF TWO NOVEL MUTATIONS IN SMIM1 RESULTING IN LOW VEL EXPRESSION

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Background: VEL negative individuals can become allo-immunized after blood transfusion with VEL positive blood or through pregnancy after carrying a VEL positive child, potentially leading to transfusion reactions or haemolytic diseases of the newborn in subsequent pregnancies. We previously found that the single transmembrane protein SMIM1 harbours the VEL blood group system. VEL negative individuals have a homozygous 17 bp-deletion (c.64-80del) in *SMIM1*. However, VEL expression levels vary due to specific polymorphisms found within the *SMIM1* gene. For instance weak VEL expression is correlated with the known single heterozygous missense mutations *SMIM1*152T>A* and *SMIM1*152T>G* in exon 4 and with SNP rs1175550 in intron 2, in which the minor G allele is associated with higher *SMIM1* transcript levels compared to the major A allele. From a clinical point of view, it is important to characterize these polymorphisms to properly evaluate the status of the VEL blood group within individuals and to design genotyping panels.

Aims: To identify novel haplotypes within the *SMIM1* gene that correlate with low VEL expression.

Methods: Dutch donors were serologically screened for VEL. Primary human pro-erythroblasts were cultured from peripheral blood mononuclear cells after isolation from whole blood and terminally differentiated [van den Akker 2010] and flow cytometry samples were taken daily. DNA was isolated from VEL weak donor PBMCs and sequenced for *SMIM1* introns and exons. RBC ghosts were made from VEL negative and VEL positive RBC for mass spectrometry analysis.

Results: Ten putative Vel-negative donors were identified by serological screening, and another donor was identified by chance. Sequencing of the *SMIM1* gene revealed in two donors novel mutations within *SMIM1*: *SMIM1*161T>C* (p.Leu54-Pro) and *SMIM1*122G>A* (p.Arg41Lys), leading to an amino acid change in the transmembrane and intracellular region, respectively. Both novel mutations are heterozygous of nature and their weak VEL expression suggests a dominant negative effect of the mutant SMIM1. We show that wild type (wt) SMIM1 is already expressed at the erythroblast stage and further upregulated during the initial stages of differentiation at the basophilic and polychromatic stage. In contrast, we found that SMIM1 expression was absent in primary cultured human erythroblasts of the donor carrying the *SMIM1*122G>A* allele, and showed only mild increase in expression during differentiation towards reticulocytes. By co-transfection of the SMIM1 wt and mutants thereof we will further investigate the mechanism underlying this dominant negative effect. Mass spectrometry using tryptic digests of VEL positive and VEL negative donors identified SMIM1 as the most deregulated protein.

Summary / Conclusions: Here we identify two novel *SMIM1* mutations resulting in very weak VEL expression in red blood cells and absence of expression at primary cultured human erythroblasts. The primary culture experiments indicate that membrane incorporation of SMIM1 is determined in the early stages of terminal differentiation and suggests that a dominant negative effect by SMIM1 mutants already occur in erythroblasts. In addition, in contrast to previous observations [Ballif et.al. 2013], we found SMIM1 to be readily detectable by Mass Spectrometry using tryptic digests.

4B-S22-03

IDENTIFICATION OF A GENE UNDERLYING THE ANWJ-NEGATIVE PHENOTYPE

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Background: AnWj (901.009) is a high-incidence blood group antigen. Red Blood Cells (RBC's) from newborns do not express or weakly express the AnWj antigen. Transition from AnWj-negative to AnWj-positive phenotype occurs 3-46 days after birth and is completed within 24–48 h suggesting addition of a substrate on existing RBC's structures. The AnWj negative phenotype and the development of anti- AnWj

may be seen secondary to transient suppression of the AnWj antigen on the patient's own RBC's in association with lymphoid malignancies and autoimmune disorders.
A rare genetic form inherited in an autosomal recessive mode was first reported from Israel in an Arab family, since then other unrelated AnWj-negative individuals were found by MDA National Blood Group Reference Laboratory (NBGRL).
The molecular basis underlying both the acquired and the inherited AnWj-negative phenotype is unknown.
Aims: To determine the genetic basis of the inherited form of the AnWj-negative phenotype.
Methods: A consanguineous Arab family with two AnWj-negative children was found by MDA-NBGRL. The parents and two additional children were AnWj-positive. Testing for the AnWj-negative phenotype and anti-AnWj were performed using standard serological methods using MDA-NBGRL rare cells and anti-sera. Three AnWj-negative, unrelated individuals of Arab descent were recruited. The study was approved by an IRB and subjects signed an informed consent.
Exome sequencing was performed on six members of the index family in search for rare homozygous variants shared by the two AnWj-negative siblings. These were considered candidate alleles and were checked in the three non related AnWj-negative individuals.
Results: Exome sequencing revealed seven candidate genes that showed complete segregation within the index family. The two AnWj-negative children were homozygous for a rare variant in all the seven genes. However, the three additional non-related AnWj-negative Arab subjects were homozygous only for one variant, rs114851602 in the *SMYD1* gene. The minor allele frequency of this variant in dbSNP is 0.002, thus the chances of an individual being homozygous for this allele are $(0.002)^2 = 0.00004$. The chances of randomly finding three consecutive such individuals are $(0.00004)^3 = 6.4 \times 10^{-14}$. To ensure its rarity in the Arab population, 100 controls of Arab origin were tested for the rs114851602 minor allele and it was not found in any of them. rs114851602 substitutes arginine for glutamine at position 320 and full sequencing of *SMYD1* did not reveal any additional changes. According to 3D protein modeling this substitution disrupts the delicate structure and function between two of the protein domains and the protein as a whole. Western blots failed to detect the SMYD1 protein in mature RBC's.
Summary / Conclusions: We present strong genetic evidence that the R320Q substitution in *SMYD1* underlies an inherited form of the AnWj-negative blood group phenotype. SMYD proteins have been found to methylate a variety of histone and non-histone targets which contribute to various roles in cell regulation such as chromatin remodeling, transcription, signal transduction, and cell cycle control. The mechanism by which the *SMYD1* mutation leads to the AnWj-negative phenotype remains to be determined.

4B-S22-04

WEAK D PHENOTYPE IN INDIANS IS PREDOMINANTLY DRIVEN BY A NOVEL DUPLICATION IN THE RHD GENE

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Background: Genetic bases of phenotypic variability in the Rh blood group system have been extensively studied in Caucasians, Black Africans and East-Asians and reported in the literature so far. Although several phenotypic studies have been conducted in Indians, little is known about the molecular determinants driving weak D phenotype in this population.
Aims: We sought to genotype those individuals 1/ to identify and characterize the genetic variations associated with weak D phenotype in Indians; 2/ to define an Indian-specific pattern of distribution of the molecular variants, and 3/ to design an Indian-specific strategy for *RHD* genotyping.
Methods: Samples of Indian origin (n = 223) were selected on the basis of weak D agglutination by standard serological testing. DNA was extracted and molecular analysis of the *RHD* gene was carried out by approaches developed at the laboratory, including 1/ Tm-shift assay for screening of weak *D* type 1, 2 and 3 alleles; 2/ Sanger sequencing for identification of base substitutions/insertions/deletions; and 3/ quantitative multiplex PCR of short fluorescent fragments (QMPSF) for assessment of *RHD* exon copy number variations (CNVs). Subsequent transcript expression analysis was carried out by standard methods, including reverse-transcription PCR, subcloning and direct sequencing when necessary.
Results: Tm-shift assay and sequencing analysis carried out initially identified only a limited number of variations in a subset of samples, including novel single nucleotide substitutions. Conversely QMPSF approach revealed a common duplication of exon 3 in a significant proportion of weak D samples (i.e. 130/223, 58.3%), suggesting a novel, predominant rare allele specific of the Indian population. Further mRNA analysis indicated a duplication of exon 3 within the transcript sequence (i.e. exons 2-3-3-4), additionally to the wild-type structure (i.e. exons 2-3-4). Very interestingly this observation suggests that: 1/ the duplicated region, including exon 3, is located between *RHD* exons 2 and 4 in a sense orientation, and 2/ a competition occurs at the transcriptional level between the biosynthesis of a wild-type, functional transcript and a variant, putatively abnormal transcript. Genomic characterization of the duplicated region finally identified a ~12 kilobase region encompassing the very 3′-end of exon 2, full intron 3, exon 3, and a partial sequence of intron 3. Subsequently we designed and successfully tested retrospectively and prospectively an Indian-specific assay for *RHD* genotyping based on a standard, multiplex PCR approach.
Summary / Conclusions: Overall, we have found a novel, predominant *RHD* variant allele specific of the Indian population. Further phenotypic characterization is currently being carried out. This discovery not only extends the current knowledge of *RH* molecular genetics, but may also have a major incidence in the process of *RHD* variant screening in this population, which accounts for about $1/6^{th}$ of the total worldwide population.

4B-S22-05

MOLECULAR STUDY OF D NEGATIVE AND D VARIANT PHENOTYPES IN ARGENTINE

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Background: The current population of Argentine is the result of generations of intermixing between Amerindians, Europeans and Africans. The contribution of these ethnic groups to the genetic pool varies in different areas of the country. In these sense, a comprehensive study of the *RH* locus in our population is needed.
Aims: The aim of this study was to investigate the *RHD* molecular polymorphism in different geographical regions of Argentine.
Methods: 796 D negative, C/E positive and 324 variant D samples from North and Central areas of the country were studied. The D, C, c, E and e status was determined by standard serologic hemagglutination techniques. DNA samples from D negative, C/E positive individuals were initially screened for the presence of intron 4 and the 3′ untranslated region of the *RHD* gene using PCR strategies. *RHD* zygosity was investigated by PCR-RFLP in those D negative samples carrying *RHD* specific sequences and in all variant D phenotypes. Allele characterization was performed by PCRs, microarray and sequencing.
Results: In the 796 D negative C/E positive phenotypes, *RHD* specific amplifications were detected in 133 samples (16.71%). Silent *RHD* alleles were identified in 12.19%, *DEL* alleles in 2.51% and no molecular polymorphisms were found in 2.01% of the samples. Among the 133 D-/*RHD*+ samples, the following silent alleles were characterized: *RHD-C-D^s* (43.61%), *RHD*581insG* (14.29%), *RHD-CE(2-9)-D* (6.02%), *RHD(329T>C)-CE(3-9)-D* (5.26%), *RHD-CE(4-8)-D* (0.75%), *RHD-CE(4-7)-D$_2$* (0.75%), *RHD(1-2)-RHD(3$_{361-371del11}$-10)* (0.75%), *RHDψ* (0.75%) and the novel *RHD*1001A* (0.75%). *DEL* alleles were: *RHD*46C* (9.03%), *M295I* (3.01%), *RHD (IVS3 + 1 g>a)* (1.50%), *RHD-CE(4-9)-D* (0.75%), *RHD*1248insG* (0.75%). In the group of the 324 variant D phenotypes, the 58.02% carried a w*eak D type 1, 2* or *3* alleles. In the rest of the samples, the following variants were found: *weak D type 4* (14.81%), *RHD*DVI* (4.63%), *RHD*DVII* (1.54%), *DFR-2* (0.92%), *weak D type 59* (0.62%), *weak D type 5* (0.62%), *RHD*DMH* (0.62%), *weak D type 15* (0.31%), *weak D type 45* (0.31%), *weak D type 48* (0.31%), *RHD*DIV type 5* (0.31%), *RHD*DVa* (0.31%), *weak D type 1/RHD-CE-D^s* (0.31%). Also 5 novel *RHD* alleles were characterized: *weak D type 93* (9.57%), *RHD*325G* (0.31%), *RHD*763A* (0.31%), *RHD*764A* (0.31%) and *RHD*911A* (0.31%). *Weak D type 93* was identified only associated to ccEe phenotype. No molecular polymorphisms were found in 5.55% of the variant D samples analyzed. It is worth mentioning that the distribution of samples carrying different *RHD* alleles varied significantly between North and Central areas of Argentine.
Summary / Conclusions: The results obtained allowed a comprehensive analysis of the *RHD* locus polymorphism in our country. To note, *weak D type 93, RHD*46C* and *RHD*581insG* alleles were most frequently found in samples of individuals from North Argentine and have not been previously reported in other populations. Considering that the Amerindian influence is greater in the North region, these *RHD* variants could be associated to Native populations. Our findings show the relevance of *RHD* genotyping for a better management of D negative units in Blood Banks

and prenatal immunoprophylaxis. Further studies are being performed in samples with no molecular polymorphisms found.

4B-S22-06

GYPB 251G>C CHANGE (SER84THR) IN GLYCOPHORIN B IS ASSOCIATED WITH AN U-LIKE ALLO ANTIBODY

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Background: Clinically significant alloanti-U is made by people with S−s−U− or S−s−U+ phenotypes associated with absence of *GYPB* exons 2-6 or with mutations, c.230C>T and c.270 + 5 g>t, affecting exon splicing, respectively. The exact molecular basis of U antigen is not known but the determinant is resistant to papain/ficin treatment. Anti-U-like (anti-GPB) antibodies have been reported in people with s+U+ phenotypes (Booth 1978 *Vox Sang* 34, Janvier 2002 *Vox Sang* 83). These antibodies primarily detect papain or ficin-sensitive determinants, but the genetic basis for antibody production has not been reported.
Aims: We performed serologic and *GYPB* investigation in two people of African ancestry, a transfused male (patient 1) and a pregnant female (patient 2) with S-s+U+ RBCs who presented with anti-U-like specificity in their plasma.
Methods: Genomic DNA was isolated from WBCs and analyzed by ID Core^{XT} (Grifols/Progenika) for Patient 1 and by PreciseType HEA (Immucor) for Patient 2. Amplification and sequencing of *GYPB* exons 1 to 6 was performed. Standard hemagglutination methods were used for antigen typing and antibody testing with licensed, unlicensed and in-house reagents.
Results: RBCs of Patient 1 typed as S−s+U + . The patient's plasma was reactive with all untreated panel cells except S−s−U− and S−s+U− Dantu+ cells and the autologous control by IgG gel test, consistent with anti-U; however, panel cells that were ficin treated no longer reacted. Patient 2 RBCs also typed as S−s+U + . Anti-U, reactive by PEG IAT and IgG gel test, but nonreactive with papain treated RBCs or with the autologous control, was identified in the plasma. By the HEA Precise Type or ID Core^{XT} analysis, both samples were *GYPB*s/s* and negative for the changes associated with either a U− or U+^{var} phenotype and predicted to be S−s+U+, consistent with the RBC typing results. To further investigate the basis of the anti-U-like plasma reactivity seen, *GYPB* was amplified and sequenced. Testing confirmed the presence of *GYPB*s/s* (c.143C/C) but also revealed homozygosity for c.251G>C (p.Ser84Thr) in exon 5. No other changes were present. Cross-testing of plasma from patient 1 was compatible with RBCs from patient 2.
Summary / Conclusions: We report here 2 people of African ethnicity with s+U+ RBC phenotypes, but who had made an antibody that resembled anti-U (anti-GPB) as only U– RBCs and the auto control were non-reactive. Both are *GYPB*s/s,* but homozygous for a c.251C change (p.84Thr). This change was previously reported, also on *GYPB*s* background (Storry 2003 *Transfusion* 43), but has not been associated with altered U/GPB. The c.251G>C is in the db SNP database (rs1132783) and homozygosity for this change has a frequency of 0.06 in Europeans and 0.005 in Africans. The amino acid at position 84 is thought to reside in the transmembrane region but the finding of an anti-U/GPB suggests the protein is altered, and may explain some of the (now decades old) observations of anti-U in s+U+ patients. While anti-U has been associated with severe transfusion reactions and HDFN, the clinical significance of the antibody made by these patients is unknown. The pregnancy of patient 2 went to term without incident.

Blood Safety: Arbovirus

4B-S23-01

HOST INFLAMMATORY RESPONSE TO MOSQUITO BITES ENHANCES THE SEVERITY OF ARBOVIRUS INFECTION

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Mosquitoes can pass disease to humans when they bite, which includes infections caused by viruses (arboviruses). Arboviruses infect many millions of people each year and include viruses that cause Zika, dengue and chikungunya. Most such infections are usually found in the tropics, but a changing climate and globalization means their range has spread at an alarming rate. Increasing numbers of tourists from e.g. Europe are also becoming infected when visiting affected areas. There are many genetically distinct arboviruses and it is hard to predict which virus will cause the next outbreak; making it challenging to develop and stockpile virus-specific medicines. In an attempt to identify common aspects of these infections that can be targeted, we have shown that mosquito bite inflammation, which is common to all such infections, enhances the systemic course and clinical outcome to infection with arboviruses. Inflammatory response to mosquito bites culminates in the entry and infection of myeloid cells in the skin. This inflammation, by inadvertently providing myeloid cells that are permissive to infection, boosts virus levels and dissemination to the blood. Furthermore, our data suggests that therapeutic intervention at the mosquito bite site / inoculation site, represents a novel strategy for targeting multiple distinct arthropod-borne viruses.

4B-S23-02

ZIKV RNA IS PRESENT AND PERSISTS FOR WEEKS IN STORED RBC UNITS FROM INFECTED BLOOD DONORS

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Background: Zika virus (ZIKV) is a Flavivirus transmitted to humans mainly by *Aedes aegypti* mosquitoes. Human infection with ZIKV was rarely reported prior to the 2007 outbreak on Yap Island in Micronesia. Since 2013, large ZIKV outbreaks have been reported in the Pacific region and the Americas, with the first mosquito-borne transmissions occurring in the U.S. in 2016. Transfusion-transmission of ZIKV has been reported in Brazil, and remains an ongoing risk to the U.S. blood supply because viremia emerges before symptoms, and approximately 80% of infected individuals never develop symptoms. While blood screening and diagnostic assays are typically performed using plasma or serum as the testing substrate, we suspected that ZIKV RNA may also be associated with RBCs, as observed for the related flaviviruses WNV and DENV. A study has shown that patient whole-blood samples were ZIKV-RNA-positive out to 58 days while the corresponding sera were positive for only 3 days.
Aims: The goal of this study was to evaluate persistence of ZIKV RNA and infectivity in RBC units from infected donors.
Methods: Leukocyte-reduced RBC units from blood donors who tested positive for ZIKV RNA in plasma on blood screening NAT assays in use under IND were obtained (Blood Systems Research Institute, San Francisco, CA and Creative Testing Solutions, Tempe, AZ) and stored in the original bag at 4°C. Aliquots were withdrawn from the units upon receipt and weekly thereafter for RNA quantitation and analysis of infectivity in tissue culture. Viral RNA, extracted from aliquots using Trizol reagent, was quantified using ZIKV-specific Taqman qRT-PCR. Tissue culture was performed by inoculating Vero cells or monocyte-derived macrophages (MDM) with RBCs for 1 h, refeeding with media and incubating at 37°C, 5% CO_2 for 7 days, with cultures monitored by qRT-PCR.
Results: ZIKV RNA was detected in RBC units from 7/9 NAT yield donations and RBC samples remained RNA-positive for at least 35 days past the date of draw (all units that were tested beyond the 42-day expiration date of the RBC units remained positive). RNA loads remained relatively constant in tested units through the course of storage. Determination of infectivity to Vero or MDM is ongoing.
Summary / Conclusions: Our study shows that ZIKV is present RBC components of donors who tested positive on NAT screening, and remain positive for the duration of storage. The mechanism of persistent ZIKV RNA in stored RBC units should be investigated with particular focus on whether RBC-associated ZIKV may be infectious under storage conditions even after resolution of detectable plasma RNA by routine donor NAT screening.

4B-S23-03

EVOLUTION OF SEROLOGICAL MARKERS IN ZIKA VIRUS NAT-REACTIVE BLOOD DONORS IN PUERTO RICO

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Background: Documented cases of Zika virus (ZIKV) transfusion-transmission and consequent risk to blood safety led to implementation of nucleic acid amplification testing (NAT) of the blood supply of the U.S. and its territories in 2016. At the peak of the 2016 epidemic in Puerto Rico (PR) over 1.5% of blood donors were reactive by ZIKV NAT by the Roche cobas® Zika NAT assay.
Aims: By enrolling NAT reactive donors into follow-up studies, together with pre-existing sample sets for dengue (DENV) and West Nile virus (WNV), we aim to study the evolution and specificity of serological responses to ZIKV.
Methods: Anti-Zika IgM was assayed using the CDC IgM antibody capture (MAC)-ELISA, while a similar capture assay was used for IgG. ZIKV and DENV reporter virus particles (RVPs) were used to study neutralization.
Results: A high degree of serological cross-reactivity occurs between flaviviruses, particularly in PR, where donors have been exposed to multiple subtypes of DENV. Using NAT-confirmed acute ZIKV, DENV or WNV donor index and follow-up samples, we studied the extent of cross-reactivity using standard serological assays. The MAC-ELISA demonstrated good specificity on WNV IgM-reactive samples (over 97%), but higher levels of cross-reactivity on DENV IgM-reactive samples (specificity of 70-85%). Anti-ZIKV IgG demonstrated an even higher degree of cross-reactivity following acute DENV infection, as well as a boosting of cross-reactive antibodies following ZIKV infection of DENV experienced individuals. Similarly, cross-reactive neutralization was observed after secondary DENV infection and ZIKV infection of DENV experienced individuals, due to stimulation of anamnestic responses. More specific neutralizing responses were observed in donors with remote flavivirus infection, suggestive of broad cross-reactivity occurring only transiently after recent infection.
During the 2016 PR epidemic, 80 confirmed ZIKV NAT reactive donors were identified from whom at least two follow-up samples, together with the index donation, were collected. At donation nearly 60% (47/80) of ZIKV NAT reactive donors in PR were seronegative; however virtually all of these donors went on to seroconvert - with 96% (77/80) conclusively seroconverting to anti-ZIKV IgM using the MAC-ELISA. Of the remaining 3 donors, the IgM result was equivocal at one time-point, suggesting a transient and blunted, but detectable, immune response. For donors seronegative at index, seroconversion had occurred by a mean of 15 days (range 5 to 59 days), while the duration of IgM responses varied greatly from less than 19 days to more than 60 days; importantly the duration and magnitude of IgM reactivity did not correlate with evidence of prior DENV immunity.
Summary / Conclusions: Despite issues with cross-reactivity, the ZIKV MAC-ELISA, particularly, is an important tool for confirmatory and diagnostic testing of recent ZIKV infection, while IgG and neutralizing responses may be more useful on long-term follow-up after cross-reactive anamnestic responses have declined.
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cobas® Zika is not commercially available for blood screening use.

4B-S23-04

AN INNOVATIVE MULTIPLEXED AND FLEXIBLE MOLECULAR APPROACH FOR THE DIFFERENTIAL DIAGNOSIS OF ARBOVIRUSES

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Background: The screening of blood donors and travelers returning from endemic/epidemic areas has highlighted the importance of multiplex diagnostic approaches for the simultaneous analysis of various pathogens. Furthermore, in the context of similar clinical signs, the differential diagnosis of arboviruses is essential to discriminate the causative agent for patient management and epidemiological surveillance. Thus, there is a real need for improving the diagnosis of acute arboviral infections. The development of a flexible diagnostic approach is a key challenge to face the continuing emergence of arboviruses, belonging to flavivirus and alphavirus, such as Dengue virus (DENV), West Nile virus (WNV), Zika virus (ZIKV), Yellow fever virus (YFV), Usutu virus (USUV) and Chikungunya virus (CHIKV).
Aims: An innovative diagnostic approach combining generic RT-PCR amplification and identification on low cost microarrays has been developed. We have patented original polythiolated probes grafted on maleimide-activated microplates for the robust, sensitive and specific detection of the amplified viral genomes.
Methods: In this study, analytical performances of the test were evaluated on viral standards and on clinical samples, previously tested by real-time RT-PCR methods: DENV (1/2/3/4 serotypes), WNV, ZIKV (Asian and African strains) and CHIKV. Forty human plasmas from blood donors with no history of contact with arboviruses endemic/epidemic areas were used as negative controls. We have designed two sets of degenerated primers for the generic RT-PCR amplification of all flaviviruses and for CHIKV. Biotinylated amplicons were captured on complementary grafted polythiolated probes on microplate. After addition of Streptavidin-Europium label, the molecular hybridization events are detected by time-resolved fluorescence using a microplate reader.
Results: One original generic probe for DENV and specific probes designed for i) each of the four DENV serotype, ii) WNV iii) the two ZIKV lineages and iv) for CHIKV, were validated. The use of our methodology combining the amplification of the viral genomes and their identification using polythiolated probes grafted on microplates shows 100% of specificity, with no false positive results on the forty control samples, and no cross reactions. Using viral reference standards, we have observed sensitivities of 1 TCID50/ml for DENV1, DENV3 and CHIKV and of 10 TCID50/mL for DENV2, DENV4 and ZIKV. Finally, the first results obtained on 110 DENV (+), 69 ZIKV (+) and 50 CHIKV (+) clinical samples show 85%, 87% and 96% correlation respectively between our approach and commercial or in house real time RT-PCR methods.
Summary / Conclusions: This innovative strategy allows the development of flexible, highly sensitive and easy to handle platforms dedicated to the multiplex screening and identification of emerging viruses. This methodology is adapted for the easy inclusion of additional molecular targets to improve the surveillance and the prevention of arboviral infections.

4B-S23-05

DETERMINATION OF ZIKA VIRUS INFECTIVITY IN PLASMA VS TISSUE CULTURE ISOLATES USING IN VITRO CELL LINES AND MOUSE MODELS

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Background: Zika virus (ZIKV) has been demonstrated to be transfusion-transmitted and to persist within various blood compartments and in particular associated with RBC. Thus, even with sensitive plasma-based nucleic acid amplification testing (NAT) in place, there remains a theoretical risk of ZIKV transfusion-transmission. Although infectivity of ZIKV is generally established using Vero cells, more sensitive detection of infectious virus may possible using animal models. Signaling through the type I IFN receptor (IFNAR) can be prevented using anti- IFNAR blocking antibodies, thus leading to a blunted anti-viral innate immune response in mice, and more efficient viral replication for many human viruses that otherwise replicate poorly in mice.
Aims: Determine relative infectivity of ZIKV in infected donor plasma vs a tissue culture isolate derived from that plasma using both Vero cell culture and a murine model system, with the ultimate aim to determine minimal infectious doses for ZIKV in various blood components over serial stages of human infection.
Methods: A ZIKV isolate was generated in Vero E6 cells from an RNA-positive plasma unit collected from an acutely infected Brazilian blood donor. This isolate was minimally passaged to produce a high titer viral stock. Using both this isolate and the primary clinical ZIKV sample, limiting dilutions were performed and analyzed for viral RNA levels, *in vitro* infectivity in plaque formation assays using Vero

E6 cells, and *in vivo* infectivity using C57BL/6J mice treated with a monoclonal antibody to block type I interferon (IFN) signaling.
Results: Limiting-dilution studies in cell culture with the ZIKV isolate demonstrated that approximately 2,500 viral RNA copies correlated to 1 infectious or plaque-forming unit (PFU). Anti-IFNAR treated mice demonstrated sustained and high titer viral replication following infection with this ZIKV isolate. A stock isolate with 4.4×10^9 RNA copies/ml yielded 1.8×10^6 PFU/ml in Vero cells and 1.4×10^7 50% infectious dose (ID_{50})/ml in the mice. Thus, the animal model is more sensitive than the culture based assay.
Using plasma from the same ZIKV NAT reactive/seronegative donor with a viral load of approximately 300,000 RNA copies/ml there were 605 PFU/ml and hence a ratio of »500 RNA copies per infectious unit. Thus, the primary clinical sample was more infectious than the associated tissue culture amplified virus. Furthermore, in the mouse model this plasma was even more infectious, with an ID_{50}/ml of 1.4×10^4 and thus a minimal infectious dose of only 21 RNA copies/ID_{50}.
Summary / Conclusions: A small animal model for ZIKV using anti-IFNAR blocking antibody was demonstrated to be more sensitive than standard tissue culture models of infectivity. Furthermore, a primary clinical sample was more infectious than the derived isolate both in tissue culture and in the *in vivo* model. A minimal infectious dose in plasma corresponding to an ID_{50} of only 21 RNA copies, indicating that ZIKV is highly infectious, which suggests that large volume transfusions of plasma from donors below the limit of detection of current NAT assays may be sufficient to transmit infection. We are now characterizing infectivity of ZIKV RNA+ RBC samples obtained from follow-up of NAT yield donors following clearance of plasma ZIKV RNA viremia.

Clinical: Clinical transfusion 3

4B-S24-01

CLINICAL AND BIOLOGICAL SIGNIFICANCE OF THE STRENGTH OF DIRECT ANTIGLOBULIN TEST: A 12 YEAR RETROSPECTIVE STUDY

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Background: Direct Antiglobulin Test (DAT) remains one of the critical tests in the assessment of Auto-Immune Hemolytic Anemia (AIHA). It is still widely used and recommended although first described more than 70 years ago. The classical grading system from negative to 4+ strength on tube technique continues to be used in our center. In the era of DAT negative AIHA, the doubt is raised about the clinical significance of the strength of positive DAT.
Aims: The aim of our study was to evaluate the clinical correlation of the grade of positive DAT (Weak, 1 +, 2 +, 3 + and 4 +) with five biological markers of hemolytic anemia (Hemoglobin level, Reticulocytes Count, Serum LDH, Unconjugated Bilirubinemia and Haptoglobin level).
Methods: Laboratory data from June 2004 to December 2016 was collected. A total of 29740 samples was available; 23781 newborn samples and 4504 adult samples with negative result were excluded. Duplicate samples from same patients were also excluded in order to avoid related bias. Finally, 866 positive results of different individuals were included and were subdivided into five groups based on the grade of DAT performed by traditional tube method:
1. Group 1 (n = 448) with weakly positive DAT (Microscopic agglutination).
2. Group 2 (n = 192) with grade 1 + positive DAT (Red cell button breakage into numerous tiny clumps visible macroscopically).
3. Group 3 (n = 105) with grade 2 + positive DAT (Red cell button breakage into medium sized agglutinates without background turbidity).
4. Group 4 (n = 62) with grade 3 + positive DAT (Red cell button breakage into several large sized agglutinates without background turbidity).
5. Group 5 (n = 59) with grade 4 + positive DAT (No red cell button breakage, one solid agglutinate is seen without background turbidity).

The groups were compared to each other based on the five biological parameters (Hemoglobin level, reticulocytes count, serum LDH, unconjugated bilirubinemia and haptoglobin level). One Way ANOVA and post-hoc pairwise comparisons (Tukey's test) were used for statistical analysis.
Results: Hemoglobin level along with reticulocyte count and haptoglobin level showed statistically significant differences between the five groups ($P < 0.05$ for all the 3 parameters), which was not the case for LDH ($P = 0.2$) and unconjugated bilirubinemia ($P = 0.6$). Further statistical analysis by pairwise comparisons revealed no significant difference for all biological parameters between lower grade groups (weakly positive, 1 + and 2 +) on one side and higher grade groups (4 + and 5 +) on the other side. However, patients of groups 4 and 5 had significantly lower levels of hemoglobin than those of group 1 ($P < 0.05$) and higher levels of reticulocyte count than those of group 1 ($P < 0.05$) and group 2 ($P < 0.05$). For haptoglobin level, although the difference was significant by analysis of variance, pairwise comparison failed to establish the same difference between the groups.
Summary / Conclusions: Our study revealed that the grade of DAT continues to be useful in the clinical settings and correlates well with the degree of hemolytic anemia especially with hemoglobin level and reticulocyte count, and to a lesser extent with haptoglobin level, although it does not significantly correlate with either LDH level or unconjugated bilirubinemia.

4B-S24-02

"TRIX" NATIONAL DATABASE OF IRREGULAR ANTIBODIES: POTENTIALLY AVOIDABLE TRANSFUSION INCIDENTS AND REACTIONS

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Background: Since 2007 the national "TRIX" database (Transfusion Register of Irregular antibodies and Xmatch problems) has been progressively implemented in the Dutch hospitals. Early in 2016, 85% of Dutch hospitals had a functional link with TRIX and full coverage is anticipated in 2017.
Registered data in TRIX include irregular antibodies, IgA deficiency, crossmatch problems and allogeneic stem cell transplantations (SCT). Hospitals, all of which have accredited transfusion laboratories, and the national blood service Sanquin may enter results. Patients are informed and are given an opt-out option. The standard settings in TRIX only show information that is entered by other hospitals on the premise that hospitals are already aware of relevant information they themselves entered in TRIX.
Aims: To demonstrate the potential for transfusion safety improvement when TRIX is fully implemented.
Methods: The TRIP (Transfusion and Transplantation Reactions in Patients) national hemovigilance office registers reports of transfusion reactions and incidents. A retrospective review was performed of reports to TRIP from 2005 to 2015 of acute hemolytic transfusion reaction (AHTR), delayed hemolytic transfusion reaction (DHTR), incorrect blood component transfused (IBCT) and other incident (OI) to assess whether they could have been avoided if TRIX had been fully operational. Reports were classified as "definitely" avoidable if irregular antibodies or other details registered in TRIX were known elsewhere but not in the hospital where the patient was transfused. Reports from transplanting hospitals concerning SCT patients, as well as reports pertaining to irregular antibodies which were known in the transfusing hospital, were judged to be "possibly" avoidable because the information, if displayed, could give an extra trigger to avoid component selection errors. Among the latter, we did not count reports where an antibody-compatible component was appropriately selected but the hospital failed to follow recommendations for preventive Rhesus and Kell compatible component selection. Reports to TRIP involving incorrect use of TRIX (TRIX incidents) were also searched for.
Results: In all, 68 reports to TRIP could potentially have been avoided if TRIX had been fully implemented (67 ICBT, 1 other incident). The annual number shows no decline. Thirty-two were "definitely" avoidable: 23 reports involved irregular antibodies detected in a different hospital and 9 concerned patients with SCT in a different hospital. In 20 cases a reaction was reported following the incident: 4x new allo-antibody, 1x AHTR, 9x DHTR, 1x mild non-hemolytic febrile reaction, 5x other reaction. There were no reports relating to IgA deficiency. In addition there were nine incidents where TRIX was not used correctly, all but one report involving failure to consult TRIX information properly. Under-reporting is likely.
Summary / Conclusions: The hemovigilance reports to TRIP demonstrate the potential for avoiding transfusion errors with the TRIX database. TRIX could provide additional support for blood component selection if hospitals opt to also show information which they themselves entered. Cases of incorrect use of TRIX underline the need for efforts to promote full implementation and correct application of the database for optimal benefits.

4B-S24-03

PRIMARY AUTOIMMUNE NEUTROPENIA OF INFANCY AND EARLY CHILDHOOD: PRELIMINARY DESCRIPTION OF THE DANISH AIN COHORT

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Background: Primary autoimmune neutropenia (AIN) is caused by anti-neutrophil auto antibodies and occurs predominantly in children younger than 3 years of age. The disease is characterized by severe neutropenia, bacterial infections and, in most cases, spontaneous resolution. The clinical importance of serological findings as well as the clinical presentation have not yet been well established.
Aims: The aim was to describe the Danish retrospective AIN cohort and the demography, immunohematological findings and clinical course of children with laboratory-verified AIN in Denmark.
Methods: All infants and children between the ages 0 and 5 years with chronic neutropenia and clinical suspicion of AIN investigated at our laboratory from 2003 to 2016 were retrospectively identified. The laboratory is the only diagnostic center in Denmark why all Danish patients were included. In 113 children, anti-neutrophil antibodies were detected using the Flow-GIFT, confirming the diagnosis. HNA genotyping was performed using a real-time TaqMan PCR assay in 83 patients and in 366 healthy controls.
Results: Male patients (55%) were slightly more prevalent than female patients. The majority of children were diagnosed before the age of 30 months (mean, 14 months). In 91% of the cases anti-neutrophil antibodies were detected in the patient's serum at the first investigation. In 10 patients (9%) repeated antibody testing with additional blood samples from the same patient up to three times at interval of 3–6 weeks was necessary for detection of the antibodies. 74% of the sera contained anti-neutrophil antibodies of the IgG class, 13% of the IgM class and 12% of the sera contained both IgG and IgM class antibodies. About 42% of the autoantibodies showed preferential binding to the HNA-1a antigen. Serological recovery predominantly occurred before 44 months (median, 20 months [95% CI:10-30]). Mild infections, particularly infection of the skin and upper respiratory tract, were more frequent than severe infections such as pneumonia, meningitis or sepsis despite severe neutropenia in most cases. The genotype *FCGR3B*01 + , *02-, *03-*, was significantly ($P < 0.000$) more frequent (0.46 vs. 0.13) in patients than controls.
Summary / Conclusions: We here describe the immunohematology and clinical findings in 113 Danish AIN patients. The autoantibodies were mainly IgG class anti-HNA-1a antibodies and the clinical course was generally mild. Interestingly, the genotype FCGR3B*01 + , *02-, *03-, expressing the HNA-1a antigen, was more frequent in our patients compared to controls suggesting a possible pathologically influence of this genotype.
This initial description of the retrospective Danish AIN cohort is in coherence with findings from mostly smaller European cohorts. Further serological and clinical data needs to be included and a prospective setting is to be designed in order to further characterize AIN in children. This will possibly lead to important new knowledge of risk factors as well as prognostic factors in childhood AIN.

4B-S24-04

DAILY PRACTICE OF RBC TRANSFUSION INITIATION AND MANAGEMENT OF SECONDARY IRON OVERLOAD IN HEMATO-ONCOLOGICAL PATIENTS IN THE NETHERLANDS: A SURVEY

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Background: Hemato-oncological patients are one of the most intensively transfused patient groups. Surprisingly, evidence-based guidelines on red blood cell (RBC) transfusion for these patients are lacking. Additionally, secondary iron overload might be an overlooked chronic complication of RBC transfusions. Iron overload has been associated with morbidity and mortality in various patient groups. The consequences in hemato-oncological patients, however, remain to be elucidated. Current guidelines on monitoring and treating such iron overload are either lacking or differ between and within countries resulting in wide variation of treating iron overload in daily practice.
Aims: To evaluate the variation in administration of RBC transfusions and management of iron overload among treating hematologist (in training) in the Netherlands. Furthermore we evaluate which clinical or decision-making factors determined their current policy.
Methods: For this cross-sectional study, all hematologists and hematologists in training in the Netherlands were asked to complete a structured, 25-question survey. A link to the web-based survey was distributed by mail to all hematologists and hematologists in training in the Netherlands. The survey distribution included one reminder, and took place between November 19, 2015 and January 26, 2016.
Results: Seventy-seven (of 325) responses were received (24%). The respondents represented all 8 university hospitals and 29 general hospitals (38%) across the Netherlands. A wide variation in hemoglobin based RBC transfusion triggers was observed, ranging from 5.6 g/dl to 9.5 g/dl. The most common trigger was 8.0 g/dl for stable in-patients and 7.2 g/dl for ambulatory patients. Commonly, two RBC units were given per transfusion episode (range 1–4). Recent cardiac ischemia and heart failure (New York Heart Association grade II-IV) were the strongest clinical factors influencing RBC transfusion policy, in order to maintain a higher hemoglobin level.
Serum ferritin was the most frequently measured iron parameter and used for the detection of secondary iron overload by 60% of the respondents. Furthermore, the height of serum ferritin was the most common reason to initiate treatment for iron overload. A serum ferritin level of 1000–1500 μg/l, 1500–2000 μg/l and 2000–2500 μg/l were the most commonly reported cut-off values with 39%, 21% and 24%.
For 81% of all respondents, a phlebotomy was the first choice of treating iron overload in case the hemoglobin level was sufficiently high. Conversely, iron chelation therapy was the first choice in 20% of the respondents. Deferasirox was the most frequently used iron chelating agent (91%), followed by deferiprone (9%) and deferoxamine (5%). A low hemoglobin level was reported as the most important clinical factor to initiate iron chelation therapy instead of phlebotomy in 87% of the respondents.
Summary / Conclusions: Our results confirm the large variation in daily practice in Dutch hospitals regarding initiation of RBC transfusion and management of secondary iron overload in hemato-oncological patients. For proper evidence-based guidelines clearly more future studies are needed.

4B-S24-05

WHAT'S THE HOLD UP? UPDATE ON DELAYED TRANSFUSIONS REPORTED TO SHOT: 7 YEARS OF DATA

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Background: A national serious incident reporting scheme in the UK noted 11 deaths and a further 83 incidents where patients were harmed by delays in emergency transfusion (2006-2010). As a result a national alert (Rapid Response Report) in 2010 advised all hospitals to review their local policies and procedures for the emergency provision of blood components and to report any such delays to the national haemovigilance scheme, serious hazards of transfusion (SHOT). The SHOT scheme collects information for delayed emergency transfusions but also for any other incidents where the patient was harmed or inconvenienced by the delay.
Aims: To review cumulative SHOT data 2010-2016 and identify the reasons for delayed transfusions and determine lessons learned.
Methods: A 7-year retrospective analysis of delayed transfusion reports submitted to SHOT (2010–2016).
Results: An increasing number of delays were reported each year (14 in the first 2 years increasing to 101 in 2016). The total for 7 years was 314 with 25 deaths related to delays in provision of red cells or platelets. The majority were urgent or emergency transfusions, 222/314 (70.7%) occurring in emergency departments, theatres and intensive care units (139/314, 44.3%). Several problems with the activation of major haemorrhage protocols were reported (n = 42) including incorrect trigger phrase, when to trigger and when to stand down. Delays were reported in 18 instances of obstetric haemorrhage including two deaths. Failures in communication between clinical areas and the transfusion laboratory were important causes of delay particularly when the laboratory staff were not made aware of the urgency. Other factors were non-availability of porters, mislabelling and other problems with samples (including wrong blood in tube events). Delays occurred due to confusion about the location of, or access to, emergency O D-negative units. Delay in recognition of serious haemorrhage occurred when patients were managed by different teams over time or transferred between departments with inadequate handover.

When transfusion was urgent but not requiring MHP activation, staff were not always clear how rapidly components can be available (so whether to request group-specific or crossmatched units), and particularly that FFP takes 30 min to thaw. Delays that inconvenienced but did not harm the patients include provision of wrong components by the Blood Service to hospitals, or components sent to the wrong destination so that patients had to return the following day.
Summary / Conclusions: Transfusion is a 9-step process where every step must be completed correctly. This is important in urgent situations, particularly in major haemorrhage where any delay including the need for repeat samples may be critical. Hospitals need to ensure that their MHPs are fit for purpose and practised by drills. Attention to detail in ordering and provision of components will also prevent delays which inconvenience patients. Handovers need to be precise but also complete.

4B-S24-06

RISK FACTORS OF TRANSFUSION ASSOCIATED CIRCULATORY OVERLOAD - A CASE CONTROL STUDY

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Background: TACO is a growing concern in transfusion. Its definition is being revised and several risk factors are suspected and listed but have not been assessed yet. In our institution the number of notifications has risen rapidly, providing enough cases to perform a case control study.
Aims: The aim was to test which risk factors are relevant and evaluate the importance of each of them.
Methods: Cases were acute pulmonary oedema caused by circulatory overload associated with transfusion (TA APO) of red blood cells (RBC), which were notified from 1 Jan 2014 to 31 Dec 2016 in our institution. After validation by the French haemovigilance network, none were classified as TRALI. As the new definition had not yet been issued, likely cases of TACO without APO were eliminated from the study. All confirmed cases were included in a case control study and controls were randomly selected among the other RBC recipients of the same day in our institution (4 controls by case whenever possible). Data were collected from hospital files of cases and controls. They comprised age, sex, weight, height, BMI, surgery and type of surgery, number of RBC plasma or platelet concentrates transfused in 24 h, chronic use of furosemide, chronic anaemia, haemorrhagic shock, chronic heart failure, left ventricular ejection fraction < 45%, atrial fibrillation, chronic heart failure, chronic respiratory failure, valve disease, high blood pressure (treated or not), end-stage and moderate renal failure.
Results: During this period of time, 42 cases of TA APO were notified for 32,523 RBC transfused to 7,512 patients and two of them died. The average rate of TA APO was 1/774 RBC in 3 years but rose steadily, 1/179 patients transfused with RBC experienced TA APO. A total of 162 controls were included in the study. Univariate analysis found that chronic furosemide treatment, hemorrhagic shock, age, valve disease, moderate renal failure, number of RBC received and high blood pressure were significantly linked to TA APO. The best logistic regression model retained only 4 independent factors: chronic use of furosemide (OR = 5.7), haemorrhagic shock (OR = 9.6), age above 85 (OR = 2.6) and high blood pressure (OR = 2.9).
Summary / Conclusions: This case control study emphasised the high incidence rate of this serious form of TACO, showed independent risk factors and assessed their importance. Some arise from medical procedures, others originate in patient's features. Being one of the first on this topic, this study needs to be confirmed by others but it might help to predict and prevent TACOs.

Resource Limited Countries: Clinical Transfusion

4B-S25-01

THE BENEFITS AND CHALLENGES OF INTERNATIONAL RESEARCH COLLABORATIONS IN TRANSFUSION MEDICINE: THE CASE OF THE FRANCOPHONE AFRICA TRANSFUSION RESEARCH NETWORK

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Globalization and internationalization are playing an increasing role in human activities and serve to promote human interaction across geographical and cultural boundaries. International research collaboration presents health researchers with opportunities to share experiences, data and methods that can provide efficient approaches to scientific and technical issues. Findings from multicenter studies may be more robust if they can be extrapolated to public health issues in different countries. The Internet, including email, teleconferencing and video-conferencing, makes international collaboration a viable and exciting reality.
Although international collaboration has great potential for facilitating the research process, it may also entail significant challenges. Differences in philosophies, culture, belief systems, values and attitudes can yield various interpretations of data collected. International partnerships in research incur financial costs related to travel for face-to-face meetings, translation and multiplication of research sites. The challenges of international research require creative problem solving and commitment to the overall purpose and benefit of the project. There is a necessity for investment of sufficient time and money to build and sustain partnerships, emphasizing also the importance of commitment by participants to the research project. Effective organization directs expertise to particular issues, and plays a key role in establishing ownership of the group process.
Francophone Africa includes 28 countries that share in common a language and similar blood safety issues even if some differences in economic development exist. There are some recently published data on blood safety in Africa but few of them have been of sufficient quality for policy decisions. This was the rationale for forming the 'Francophone Africa Transfusion Research Network' with the objective of developing common evidence-based blood safety policies which may be adapted to each country's situation. The network was created in May 2007, during the first transfusion safety from infectious disease course organized jointly by the Institut Pasteur in Paris, the Institut National de la Transfusion Sanguine in Paris, France and the Blood System Research Institute (BSRI) San Francisco, United States. This group, consisting of researchers from African blood services, conducts collaborative research and promotes transfusion safety in Africa at the local, national and international levels, and proposes appropriate and comprehensive policy responses. Completed projects are based on data collected by local researchers who contribute their effort, along with some financial support and coordination by the INTS and BSRI. As of December 2015, the group included 137 researchers from 52 transfusion centers distributed in 22 countries. The Group's activities have focused mainly on epidemiological and laboratory research on blood transfusion and on suggesting blood safety strategies, particularly in the field of Transfusion Transmitted Infections (TTIs).
This network may be expanded to other African countries or similar networks may be created, all with the goal of increasing the availability of relevant data to develop and monitor appropriate strategies for blood safety in Africa.

4B-S25-02

BLOOD CELL STORAGE UNDER USUAL CONDITIONS IN AFRICA – A USER'S PERSPECTIVE?

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Background: Transfusion of blood is a life-saving intervention and an essential part of any health system. Quality-assurance practices are a legal requirement for blood transfusion services (BTS) in high-income countries and minimize patient risk. Yet, even within this context the prolonged storage of donor blood remains controversial and transfusions given to critically ill patients have resulted in unintended (adverse)

consequences. In sub-Saharan Africa (sSA) demand for transfusion is high, with most being given as emergency interventions, yet little research has been conducted to inform us about the quality and safety of donor blood or its effect on outcome on outcome due to the lack of relevant research. Prior to the adoption of PEPFAR policies most hospitals in sSA predominantly issued whole blood, which required limited processing time and manpower. Implementation of PEPFAR recommendations, including exclusive use of component preparation, has resulted in a more diverse range of blood pack types. The impact of these challenges on the quality of care given to patient's receiving transfusions and outcome of a transfusion are yet to be quantified and documented in the African setting where transfusions are used as emergencies and not pre-planned as is the case with the developed countries.
Aims: To describe the quality of blood in use in sSA.
To investigate potential mechanisms that may affect the quality of donor blood used for transfusion and that may ultimately influence short-term and longer-term (6-month) clinical outcome.
Methods: The conduct of a GCP-compliant multicenter clinical paediatric trial Transfusion and Treatment of Severe Anaemia in African Children: a randomized controlled Trial (TRACT; ISRCTN 84086586) provided an opportunity to describe the proportion of blood-pack types, haematological quality of blood and storage times (from donation to transfusion of the blood) of donor blood routinely supplied for transfusion by the Ugandan and Malawian national and regional BTS.
Results: This study will highlight the challenges faced and lessons learnt in ensuring availability of well-characterized donor packs in a clinical trial setting.
Summary / Conclusions: This study will hopefully contribute to improvements in the storage and use of blood that will result in the more effective management of this limited resource, improve patient survival post-transfusion, and reduce the need for re-transfusion and consequent shortages.

4B-S25-03

THE EVIDENCE (OR LACK OF) CONCERNING PACKED CELLS VS WHOLE BLOOD IN LOW AND MIDDLE INCOME COUNTRIES (LMIC)

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Background: Blood transfusions in resource-poor settings are frequently given as whole blood but there is little evidence to guide whether packed red cells should be used instead of whole blood. Although WHO advocates the use of packed red cells, national African guidelines are ambiguous regarding the clinical justification for whole blood or packed red cells. Evidence from more wealthy settings cannot be extrapolated to resource-poor settings because of differences in clinical needs and blood service systems.
Aim: This systematic scoping review identified and summarised evidence for giving packed red cells or whole blood transfusions in obstetric haemorrhage, pregnancy-related anaemia, and severe paediatric anaemia, in Sub-Saharan Africa (SSA). These conditions are among the most important indications for transfusions in SSA, so clinical evidence favouring either whole blood or packed cells could significantly impact African clinical transfusion guidelines and blood service organisation.
Methods: Two independent reviewers screened the results of a search strategy that covered Medline, Cinahl, Global Health, Cochrane library, and NHSBT Transfusion Evidence Library to select those that met pre-determined criteria full text review. Information from full text studies was mapped onto a pre-designed matrix and the information was summarised.
Results: Thirty-three of 11,234 publications underwent full text review; one met the criteria for inclusion. This was a single centre retrospective study comparing whole blood with packed red cell transfusion in post-partum haemorrhage. No studies comparing whole blood with packed red cell transfusion for paediatric or obstetric anaemia were identified.
Conclusion: There is very little evidence comparing whole blood with packed red cell transfusions for common clinical indications for transfusion in SSA. It is therefore unclear whether policies regarding use of these resources for transfusion are appropriate. Building a relevant evidence base is necessary in order to develop policies promoting the most appropriate use of blood in African settings.

Immunobiology of blood cells: Platelets

4C-S26-01

PLATELET IMMUNOBIOLOGY

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The immune response against platelets is a complex process involving many aspects of the innate and adaptive immune system. Immune destruction of platelets and megakaryocytes and the thrombocytopenia that ensues can cause different clinically significant haematological disorders such as transfusion-induced platelet refractoriness or immune thrombocytopenia (ITP). These conditions are frequently difficult to manage so an understanding of the biological nature of these adverse conditions is critical for an understanding of how to potentially reduce them. Superimposed on these immune platelet attack mechanisms are the immune characteristics of the platelets themselves. This lecture will give an overview of how the immune system recognizes platelet antigens and mounts efficient effector mechanism to mediate thrombocytopenia. It will also present new evidence to suggest that platelets also play a role in stimulating these responses and suppressing them; a scenario where the prey becomes the predator. The session will leave the audience with an appreciation of the various mechanisms of platelet immunity and attack and how they lead to serious clinical disorders.

4C-S26-02

ROLE OF EXTRACELLULAR VESICLES IN PLATELET TRANSFUSION MEDICINE

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On activation or apoptosis, platelets shed submicron vesicles known as microparticles, also called microvesicles. As any other cellular lineages may also produce extracellular vesicles, microparticles are heterogeneous on the basis of their cellular origin. Recent studies further demonstrate that microparticles derived from platelets harbour different surface markers, and that some of them can also convey functional organelles, such as mitochondria. Extracellular mitochondria, which are recognized damage-associated molecular patterns, were suggested to contribute to inflammation. Interestingly, several studies confirmed extrusion of mitochondria during platelet isolation, storage, and treatment with pathogen inactivation systems. Microparticles and extracellular mitochondria can interact with cells in transfused recipients, and their accurate detection may reveal distinct roles for these subtypes of microparticles in transfusion medicine.

4C-S26-03

EXPLORING NEW AVENUES FOR COLD STORAGE OF PLATELETS

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The use of platelet transfusions has increased dramatically in the last decades, but a safe, long-term platelet storage method remains missing. Current practice has platelets stored at 20 to 24°C after preparation, which has a limited lifetime up to 4–6 days primarily due to concerns about bacterial contamination. Refrigerated storage reduces platelet life-span because it causes glycoprotein-Ib (GPIb) receptors to cluster on specific microdomains of the platelet membrane. Recognition by host of specific de-glycated/de-syalylated residues on clustered glycoproteins (GP) results in platelet phagocytosis and clearance. Thus, prevention of GP clustering through

targeting of the biochemical mechanisms represents a useful target for chemical intervention. Platelet glycoproteins are intimately associated with intracellular cytoskeleton and their glycosylation depends on the location and activity of specific glycosyl-transferases. GP clustering depends on the formation of lipid rafts in the platelet membrane which in turn depends on the dynamics of the highly regulated processes of cytoskeletal rearrangements. RHOA and RAC1 form part of the RhoGTPase family of GTP-binding enzymes that are central regulators of cytoskeletal rearrangements, and have been shown to control lipid raft formation and composition. Therefore, changes in Rho GTPase activities may influence platelet membrane lipid raft assembly and glycoprotein composition. Our data using genetic and pharmacological means show that cold receptor activation stimulates Rho GTPase activities which in turn regulate platelet activation by diverse agonists, influence platelet membrane microdomain assembly, and control glycoprotein composition/clustering. We also found that murine, human and Rhesus-macaque washed platelets cold-stored in the presence of Rho GTPase inhibitors, RHOA inhibitor in particular, can circulate at levels similar to room-temperature stored, control platelets and maintain their hemostatic function in vitro and in vivo. Our data also demonstrate that washing of platelets stored for 7 days in RHOA inhibitor/plasma maintains collagen–induced shape change as well as normal aggregation of human platelets and restores bleeding time correction after congenic or autologous transfusion in all aspirinated mice and 80% of aspirinated Rhesus monkeys, respectively. The mechanism of action of G04 seems to be related to its activity blocking the process of intracellular traffic of GP through lipid rafts and endocytotic intermediates as assessed by confocal microscopy of GpIb and the vacuolar sorting protein VPS33b, as well as biochemical fractionation of detergent-insoluble membrane lipid rafts, resulting in reduced blebbing and formation of microparticles upon storage in RHOA inhibitor/plasma. Our data support that reversible inhibition of RHOA allows the extended cold storage of platelets which are effective in vitro and in vivo, suitable for use in clinical safety and efficacy trials in thrombocytopenic and/or bleeding patients.

Donors/Donation: Donor Health

4C-S27-01

"YOU SAVED A LIFE": HOW PAST DONATION USE INCREASES DONOR REACTIVATION VIA IMPACT AND WARM GLOW

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The need for reactivation strategies is high as donors are difficult to retain. For example, data from the German Red Cross Blood Donation Services show that drop-out rates or active donors may go up to 17.8%.

We propose that informing lapsed donors of their past donation use influences reactivation behavior positively, and that this effect is transmitted through a serial mediation via donation impact and warm glow. In a field experiment conducted in cooperation with the German Red Cross, we investigate the effectiveness of past donation use on reactivation behavior. We compare the influence of appeals based on past donation use to a best-practice mailing which simply acknowledges the past donation, as well as to a mailing based on future donation use. The results indicate that providing information on past donation use increases the probability of re-donation compared both other settings.

In two online studies we replicate this effect for blood and monetary donations and investigate the psychological transmission mechanism behind it. We propose and demonstrate that the influence of past donation use on the intention to re-donate is transmitted through increased warm glow. Specifically, the transmission is a serial mediation, in which past donation use increases the perceived donation impact, then induces warm glow which translates into a higher intention to donate in future. Our results support the serial mediation for blood and monetary donations.

Our work provides much needed academic evidence and our results are relevant for managers and researchers alike. Our work contributes to the literature on donor reactivation, and more generally to literature on pro-social behavior. With respect to donor reactivation, we start first by linking literature on warm glow and donation impact with the literature on lapsed donor reactivation to show that warm glow is an important driver of donor reactivation. Specifically, we show that for donor reactivation purposes, it is not sufficient to merely incorporate personalized information from the past donation history, or acknowledge a past donation. NPOs should rather incorporate information that is able to increase the perceived donation impact, and the warm glow. Second, we add to the theoretical understanding of how different types of reactivation appeals work, and expand the literature on how donation appeals can be utilized for reactivation strategies.

With respect to the literature on pro-social behavior, this study adds to the current stream of literature on the role of warm glow in pro-social processes. Current studies have shown how warm glow mediates service satisfaction for participants in a green program (Giebelhausen et al. 2016). We show that warm glow is an important motivator of re-donation behavior that mediates effect of reactivation campaigns and underline the importance of warm glow for pro-social behavior. In addition, our findings are also relevant for literature on recognition effects (Winterich et al. 2013). We show how recognition should be framed in the reactivation context and describe the underlying process.

Besides the academic relevance, our work is highly relevant for NPO managers. Our proposed strategy, which informs lapsed donors of their past donation use can be easily implemented by NPOs. Many NPOs rely on donor relationship systems, so that information from past donation use is easily available. In fact, blood donation services in Sweden have implemented this strategy (although not exclusively for lapsed donors) and inform donors on the use of their last donation.

4C-S27-02

POSITIVE AND NEGATIVE EFFECTS OF BLOOD DONATION

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Background: Although donation of blood occasionally has an immediate negative influence on some blood donors, other donors express a feeling of increased energy and a general increase in wellbeing after donation. Some donors even report that they experience symptoms indicating that "it is time to donate blood again", which might manifest as a feeling of unease or headache, which is relieved after donating blood.

Aims: The aim of this study is to determine the symptoms and frequency of the Danish blood donors experiencing positive and negative effects of blood donation, and to study possible associations with gender, age, BMI, smoking status, hemoglobin, and the donation frequency.

Methods: A questionnaire was developed with the intention of covering the most frequently experienced symptoms in relation to blood donation. The questionnaire contained eight questions about physical and psychological effects related to blood donation. By a five point Likert Scale, ranging from *always* to *never* or *excellent* to *poor*, donors were asked to indicate if they experienced the present symptom prior to and/or after the blood donation. The questions were incorporated in the ongoing electronic questionnaire in the Danish Blood Donor Study and data collection took place from March to June 2016.

Returning blood donors visiting a Danish Blood Collecting Center were asked to fill in the questionnaire and provided a written consent for subsequent data extraction from Danish Health Registers. Logistic regression analysis was used to study associations between donor characteristics and experienced effects of blood donation.

Results: A total of 2445 donors aged 18–66 years were included in the study (56% males and 44% females). Overall, comparing symptoms experienced prior to blood donation with symptoms experienced after blood donation, results reveals that 60% of the donors experienced one or more effect of donating blood. Among these donors, 19% experienced only positive effects, 28% only negative effects and 13% experienced both positive and negative effects.

The most notable positive effects of blood donation were alleviated headache (14%), feeling lighter (15%), less tiredness (7%), more energetic (5%), and less unease (5%). The most notable negative effects were more dizziness (20%), more fatigue (21%) and less energy (23%).

Logistic regression analysis revealed that the positive effects were more likely to occur in donors with higher BMI and among smokers. Negative effects were more likely to occur in female than in male donors and were more likely to occur in donors at lower age and BMI.

Summary / Conclusions: The data shows considerable both positive and negative perceived effects of blood donation. Gender, BMI, smoking status and age seem to be characteristics associated with these effects.

The number of donations and the donation frequency does not seem to be associated with either positive or negative effects, indicating that effects of blood donation do not influence the donor return rate.
By identifying relevant negative and positive effects in conjunction with blood donation and associated factors, donor health care and donor information may improve.

4C-S27-03

SO JUST HOW HEALTHY ARE OLDER AUSTRALIAN BLOOD DONORS? EXAMINING THE HEALTHY DONOR EFFECT

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Background: To donate blood in Australia, one must be knowingly healthy. Stemming from the eligibility and safety selection criteria for donating blood in Australia, the resulting cohort of donors are mostly healthy individuals. To understand the sustainability of long-term 'career' donors, it is essential to evaluate the impact of blood donation on longer-term health outcomes. However comparing against the general population is inappropriate due to the selection bias induced through the screening process. Therefore one needs to identify an appropriate comparator group, and mitigate this selection bias known as the Healthy Donor Effect (HDE). There is little known about how-to select a comparator group from the general population of non-donors.
Aims: The aim of this analysis is to examine the extent of the 'healthy-donor' selection effect in older Australian blood donors (≥45 years old), and explore novel methods for identifying appropriate comparator groups from the general population of non-donors.
Methods: Analyses were based on data collected from a large-sample (N = 267,005) longitudinal comprehensive health-survey, The Sax Institute's 45 and Up Study. The survey covers a broad set of questions aimed towards capturing aspects of 'healthy ageing' including detailed questioning on mental health, physical limitations, chronic illnesses, current health-related treatments, diet, and overall general health and wellbeing.
Stratified by sex and age groupings, survey responders were classified into latent health-states using a k-means clustering algorithm. The clustering was based on the indicated severity of responses to survey questions covering 9 health dimensions including mental, physical, comorbidities, treatment, vision, memory, dental, and self-rated overall health. After establishing a latent health-state for all survey participants at baseline, we identified blood donors using a question from the follow-up survey administered 5-years after the initial survey. We examined the distribution of blood donation within each age-sex-health cluster stratum, and then compared follow-up survey responses for self-rated overall health in donors and non-donors.
Results: A total of 237,407 (89%) participants were allocated to 1-of-5 different health-states by the clustering algorithm. Of the participants aged 55–65 years at initial survey completion (n = 56,651), a total of 17,249 were allocated to the *healthy* cluster, and 20% of these were reported recent blood donors (adjusted for non-response follow-up). Similarly, for those allocated to the *un-healthy* group (n = 5,355), a total of 6% were recent blood donors. We report a 4-fold increase (3.8 [95% CI: 3.2, 4.4]) in odds for recent blood donation for healthy vs. un-healthy populations. In the *healthy* cluster, the odds of self-reported overall health of '*excellent/very good*' vs. 'good/fair/poor' were 1.4 [95% CI: 1.2, 1.6] for recent donors, compared to non-donors, and similarly 2.3 [95% CI: 1.6, 3.2] in the *unhealthy* cluster.
Summary / Conclusions: Matching our expectations, there were a higher proportion of recent blood donors in the identified *healthy* clusters. Interestingly, within this group, there was still a significant difference in overall self-reported health between recent blood donors and non-donors. Ideally, a smaller odds ratio might indicate that a priori health-state clustering can adequately grouped like-for-like donors and non-donors, and thus allow for appropriate comparisons. Our results suggest that further statistical methods (i.e. propensity score adjustment) may be required to further reduce the healthy donor effect selection bias.

4C-S27-04

PREVALENCE, ASSOCIATED FACTORS AND DETRIMENTAL LINKS TO RESTLESS LEGS SYNDROME IN DANISH BLOOD DONORS

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Background: Restless legs syndrome (RLS) is a neurological sensorimotor disorder characterized by an urge to move the legs due to uncomfortable sensations. It is suggested that RLS is linked to sex, age, socioeconomic status, lifestyle, and iron deficiency. Blood donors are at increased risk of iron deficiency with donation intensity as the strongest predictor. RLS symptoms are linked with dysfunction in brain iron and in dopaminergic systems and they increase in intensity in the evening and at night making both sedentary activities and sleep difficult. It is therefore likely that RLS associates with poorer health-related quality of life (HRQL) and depressive disorder.
Aims: To estimate the RLS prevalence and the link to donation intensity and to identify associated sociodemographic, economic, and lifestyle factors. Moreover, to investigate the association between RLS and the mental and physical component scores of the 12-item Short form Health Survey (SF-12) (MCS and PCS) and depressive disorder, respectively, in a population of blood donors.
Methods: Cross-sectional cohort study with 12,822 Danish blood donors enrolled in the Danish Blood Donor Study (DBDS) from May 2015 to May 2016. Data on RLS was collected using the validated Cambridge-Hopkins RLS questionnaire. Donation history was obtained from digital blood bank systems. Information on depressive disorder and HRQL levels was scored using the Major Depression Inventory and the SF-12, respectively. We obtained lifestyle, socioeconomic and demographic data from a survey and from National Population Registers. Data was linked at the individual level using civil registration numbers. Linear and logistic regression models were applied.
Results: 7.2% female donors and 4.5% male donors were classified with RLS, while 12.2% of the women and 8.5% of the men reported RLS-related symptoms. RLS and related symptoms associated with (odds ratio, 95% confidence-interval): donation frequency in women with high intensity female donors having increased probability of RLS (1.22, 1.04–1.43), female sex (1.65, 1.42–1.29), higher age in women (1.60, 1.15–2.24), smoking (women: 1.40, 1.08–1.81) (men: 1.43, 1.16–1.75), frequent alcohol consumption in men (2.06, 1.24–3.42), low education (women: 1.25, 1.02–1.54) (men: 1.34, 1.06–1.71), parity (1.26, 1.07–1.48), and obesity (women: 1.34, 1.16–1.57) (men: 1.21, 1.02–1.44). Moreover, RLS linked with poor MCS in all donors (women: 1.62, 1.20–2.19) (men: 1.48, 1.06–2.07) and with poor PCS in men (1.95, 1.45–2.66), and with increased probability of depressive disorder in both women and men (women: 1.74, 1.18–2.56) (men: 2.53, 1.60–4.01). Poor quality of sleep was associated with lower MCS and depressive disorder among RLS cases.
Summary / Conclusions: RLS is common among blood donors and associated with substantially reduced HRQL and increased depression. We showed an association between RLS-related symptoms and donation intensity 3 years prior to RLS-assessment in female, but not in male donors, indicating that RLS might link to reduced iron. Our findings make it possible to detect donors with a higher probability of having RLS. Moreover, because of the modifiable nature of some of the associated factors, the present results can be applied to RLS prevention.

4C-S27-05

PLATELETPHERESIS EFFICIENCY: COMPARISON OF FOUR DIFFERENT AUTOMATED CELL SEPARATORS

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Background: The availability of single donor apheresis platelet has been a major impact in management of patients with low platelet count. There are various automated cell separators available for collection of platelet by apheresis. Therefore selection of an efficient equipment is of utmost importance.
Aims: To evaluate four automated cell separators used for plateletpheresis on the basis platelet yield, volume processed procedure time, adverse reaction and collection efficiency.
Methods: A prospective analysis of 931 plateletpheresis procedure was done which were performed using four different cell separators (Fresenius COM.TEC, Fenwal Amicus, Haemonetics MCS+ and Trima Accel). The procedures were performed as per departmental protocol. The target platelet yield for all procedures was taken as 3×10^{11}/ bag and all the procedure analyzed were single venous access. An informed consent from donors before the procedure and ethical clearance from the institute board was taken prior to study.
Results: The mean platelet yield was significantly higher for Trima Accel ($3.45 \pm 0.45 \times 10^{11}$/bag) in comparison to Fresenius COM.TEC ($2.95 \pm 0.86 \times 10^{11}$/bag), Amicus ($3.05 \pm 0.43$ X10^{11}/bag) and Haemonetics MCS+ ($2.88 \pm 0.42 \times 10^{11}$/bag). The collection efficiency of the Trima Accel (69.8 ± 6.88%) was significantly better in comparison to Fresenius COM.TEC (64.94 ± 5.86%), Amicus (66.1 ± 4.76%) and Haemonetics MCS+ (60.2 ± 5.84%). The time taken for completion of procedure was highest with Haemonetics MCS + (69.8 ± 12.1 min) than Fresenius COM.TEC (57.1 ± 7.3 min), Amicus (55.8 ± 5.2 min) and Trima Accel (53.40 ± 6.2 min)
There was a significant fall in hematocrit post procedure but no significant difference on comparison of all four cell separators. Similarly there was no significant difference in volume processed.
Of the total 931 procedure, adverse event was seen in 229 procedure with symptoms related to citrate toxicity were the most common (n = 188,). There was no correlation found between the type of cell separator used and occurrence of adverse events.
Summary / Conclusions: It was observed that all the cell separators collected platelets efficiently. Though the collection efficiency and platelet yield was significantly better in Trima Accel in comparison to the other three. There was no significant difference in terms of occurrence of adverse events, fall in hematocrit post procedure and volume processed. Haemonetics MCS+ took the longest time for completion of procedure.

Blood Safety: New methods

4C-S28-01

TOTAL LABORATORY AUTOMATION IN BLOOD DONOR SCREENING – HOW MUCH IS POSSIBLE AND USEFUL?

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Background: Since the middle of the last century, when blood grouping structures were firstly described by Karl Landsteiner, screening laboratories were characterized by manual systems. Screening was done only for a few parameters (e.g. AB0 blood typing, Rhesus antigens, HBsAg) with a huge number of employees. In the next level (laboratory 2.0) high throughput instruments were developed. The work in a screening laboratory was divided into "hands on time" and "work alone time" of the instruments. This level is currently the situation in many blood donor services worldwide and includes testing for nucleic acid tests (NAT), serology, clinical chemistry and blood typing.
Aims: In the next level of automation all procedures in a screening laboratory (pre-analytic procedures, analytic procedures and post-analytic procedures) can be merged together by automated track systems. This will be an improvement to the next laboratory level (laboratory 3.0). In brief, samples will be placed into a bulk sorter, following by an automated centrifugation, an automated decapping, building of aliquots, automated processing to several analyzers and an automated archiving of sample tubes. Re-testing of initial reactive samples in duplicate or a mini-pool deconstruction can be done by initiating of a new electronic job without any "hand on time" by technicians. The complete automated screening laboratory is feasible by harmonizing the pre-analytic condition and by reducing test volume for screening tests to a minimum.
Results: A pre-analytical study demonstrates that under moderate centrifugation conditions, NAT testing, serology testing, clinical chemistry testing as well as blood typing can be done from one sample tube without reduction in specificity and sensitivity. The implementation of an automated track system which combines pre-analytical processes, the analytical processes and post-analytical processes is able to reduce the total number of employees to a minimum and to enable a 24/7 performance in a laboratory.
Conclusions: The next level of laboratory automation (laboratory 3.0) is now available by different manufacturers and enables a continuous sample workflow in the laboratory. Manufacturer have now more than 10 years experience with track systems and try to integrate into their systems all parts for blood donor screening. This new level will reduce personnel costs to a minimum and increase laboratory flexibility especially for special seasons of the year like Christmas or Easter holidays to a maximum and will increase blood safety in order to exclude manual failures.

4C-S28-02

BIOFILM MATRIX AND CELL WALL MODIFICATION DURING BIOFILM FORMATION MIGHT CONFER STAPHYLOCOCCUS EPIDERMIDIS ADVANTAGEOUS GROWTH IN PLATELETS

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Background: Bacterial contamination of platelet concentrates (PCs) poses the greatest post-transfusion infectious risk. *Staphylococcus epidermidis* is the aerobic bacterium most frequently isolated from PCs, where it forms matrix-embedded aggregates (biofilms), increasing the risk of missed detection during PC sterility testing. A typical *S. epidermidis* biofilm matrix is composed of polysaccharide intercellular adhesin (PIA), however some strains produce a matrix containing mainly proteins and extracellular DNA (eDNA). Our previous studies have shown that the PC storage environment triggers *S. epidermidis* biofilm formation, even by strains traditionally considered to be biofilm-negative. It has been reported that the bacterial biofilm matrix composition varies in different environments and the bacterial cell wall undergoes remodeling during biofilm formation.
Aims: This study was aimed at characterizing changes in the biofilm matrix composition and the structural pattern of the peptidoglycan, a major cell wall component, of *S. epidermidis* strains grown in PCs.
Methods: Three biofilm-positive *S. epidermidis* strains (ST10002, AZ39 and AZ22) and a biofilm-negative strain (ST11003) were used herein. Biofilms were grown in PCs (5 days/20-24°C/agitation) or in glucose-supplemented Trypticase Soy Broth (TSBg) (24 h/37°C/static). Production of PIA was assayed by immunoblot (N = 2). The proteinaceous and eDNA composition of the biofilm matrix was evaluated by disruption assays using proteinase K and DNase I, respectively (N = 3). The peptidoglycan profile of *S. epidermidis* ST10002 and AZ39 biofilm cells grown in TSBg and PCs was determined by HPLC (N = 4). MALDI-TOF and tandem mass spectrometry (MS/MS) were used to determine the chemical composition of selected HPLC peptidoglycan muropeptides from biofilm cells.
Results: In TSBg, a PIA-based biofilm matrix was detected in *S. epidermidis* ST10002 while AZ39 and AZ22 produced a protein-based matrix. The biofilm matrix of the three strains also contained eDNA. *S. epidermidis* ST11003 did not produce biofilms in TSBg; however, it converted to a biofilm-positive phenotype in PCs. The biofilm matrix of the four strains grown in PCs was predominantly of a proteinaceous composition. MALDI-TOF analysis of five HPLC selected peptidoglycan muropeptides from biofilms grown in TSBg revealed that all of them were D-Glu amidated and one was also O-acetylated. MS/MS analysis showed identical chemical composition of four of the five muropeptides between strains ST10002 and AZ39. Preliminary MALDI-TOF and MS/MS analyses of the peptidoglycan from *S. epidermidis* biofilms grown in PCs also show the presence of D-Glu amidated and O-acetylated muropeptides. Interestingly, peptidoglycan of biofilms from PC cultures has shorter muropeptides and reduced serine content.
Summary / Conclusions: Platelet storage induces structural changes in the *S. epidermidis* cell wall and biofilm matrix composition. The biofilm matrix of *S.*

epidermidis grown in platelets is mainly of proteinaceous nature. Peptidoglycan amidation and O-acetilation are involved in increased resistance to lysozyme and antimicrobial peptides in staphylococci. Therefore, the structural changes observed in our studies are likely responsible for *S. epidermidis* resistance to platelet immune factors conferring an advantageous growth to this microorganism in the platelet storage milieu. Investigation of bacterial physiological changes induced during platelet storage is important to propose new strategies to reduce bacterial contamination and enhance platelet safety.

4C-S28-03

MALDI-TOF MASS SPECTROMETRY: A NOVEL METHOD FOR THE DETECTION OF BACTERIAL CONTAMINATION IN PLATELET CONCENTRATES

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Background: The use of unstable blood products is associated with a very high level of safety today. However, bacterial risks still exist, more particularly for Platelet Concentrates (PCs) given their preparation and storage conditions.
Although many techniques for detecting bacterial contamination in PCs are proposed, there is currently no rapid, sensitive and specific reference method. It is the cultivation methods, in particular the BACTEC and BacT/ALERT methods, which are the most frequently used.
We have developed a rapid and sensitive method for detecting microbial contamination of samples from PCs by Matrix-Assisted Laser Desorption Ionization Time-Of-Flight Mass Spectrometry (MALDI-TOF).
MALDI-TOF has recently emerged as a powerful and rapid tool for routine bacterial identification of bacterial colonies from cultures "La Scola, 2009, PloS One".
Its principle is based on the recognition of peptide spectra; the bacterial identification is carried out by spectral homology linked to a database supplied by the manufacturer.
To our knowledge, this is the first time that MALDI-TOF has been used to detect any bacterial contamination in PCs which are considered a complex environment.
Aims: We have compared the sensitivity, the specificity and the speed of the BACTEC cultivation method to the MALDI-TOF approach that we developed.
Methods: The PCs were contaminated in order to obtain a final concentration of 10^2 CFU/bag at the time of inoculation. Seven strains were tested. For each strain, the tests were performed on 5 PCs. After inoculation, the PCs were left under stirring for 24 h at room temperature. At the end of this period, 8 ml of contaminated PCs were taken from a BACTEC flask and in parallel 1 ml of PCs was incubated for 8 h at 37°C with stirring in the presence of trypticase soy broth in order to be analyzed by MALDI-TOF. These contaminated samples underwent a step of debridement with saponin followed by extraction with formic acid and acetonitrile. The spectra were analyzed by the MALDI Biotyper software version 3.0 (Bruker Daltonics). Each bag was analyzed 30 times by MALDI-TOF, for a total of 1050 tests.
Results: The analysis of the samples by MALDI-TOF allowed for the detection and early identification at 8 h of all bacterial strains tested on 35 contaminated bags of PCs as well as an identification of all the bacteria tested.
Analysis by BACTEC of PCs infected with *Escherichia coli* and *Providencia stuartii* allowed identification at 04 h25 $\pm$ 0.03 and 06 h38 $\pm$ 0.040 respectively.
For the remaining bacteria, the detection time by BACTEC was significantly longer than 8 h (mean: 10 h41, n = 25, P value <0.005, T-test).
Summary / Conclusions: We have demonstrated the possibility of detecting bacteria in the PCs with MALDI-TOF, whatever the strain and very quickly by obtaining the results for the detection at 8 h after 24 h of agitation, with sensitivity and specificity comparable to that of the BACTEC method.

4C-S28-04

PATHOGEN INACTIVATION OF ZIKA, DENGUE AND CHIKUNGUNYA VIRUSES IN ALL BLOOD COMPONENTS

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Background: Pathogen inactivation (PI) is a proactive mitigation strategy available to safeguard the blood supply from the threat posed by emerging infectious agents like Zika virus (ZIKV). The blood bank industry recently reacted to the risk associated with documented ZIKV transfusion-transmitted infections (TTIs). TTIs have been reported for other arboviruses including all dengue virus (DENV) serotypes but not for chikungunya virus (CHIKV). However accidental laboratory infections from infectious samples and the detection of CHIKV RNA-positive blood samples from asymptomatic donors confirmed the risk for CHIKV TTIs.
Donor screening is not sufficiently effective to reduce risk as most DENV and ZIKV infections are asymptomatic. Licensed arbovirus NAT assays are not available in endemic areas and NAT assays, even if multiplexed, cannot detect all arboviruses that may co-circulate in tropical and subtropical countries. PI on the other hand is a proactive strategy designed to inactivate pathogens in blood components by inhibiting nucleic acid replication, transcription, and translation. Licensed PI systems are available for plasma and platelets and a PI system is in development for red blood cells (RBCs).
Previous published studies reported the inactivation of DENV and CHIKV to the limit of detection using amotosalen and ultraviolet A light (S-59/UVA) for the treatment of plasma and platelets. Moreover, data have been reported for the inactivation of ZIKV to the limit of detection in plasma using S-59/UVA and in RBCs after chemical treatment using amustaline and glutathione (S-303/GSH).
Aims: We investigated the efficacy of S-59/UVA to inactivate high level of ZIKV titers in platelets and the efficacy of S-303/GSH to inactivate high level of DENV and CHIKV in RBCs.
Methods: Platelet units were spiked with high levels of ZIKV titers and RBCs units were spiked with high levels of DENV and CHIKV titers. For each virus, infectivity levels (expressed in $\log_{10}$ $TCID_{50}$/ml) and RNA loads (expressed in $\log_{10}$ Geq/ml) were measured before and after PI treatment using previously published methodology.
Results: S-59/UVA inactivated > 4.4 $\log_{10}$ ZIKV $TCID_{50}$/ml (corresponding to 7.5 $\log_{10}$ Geq/ml) to the limit of detection in platelets; S-303/GSH inactivated > 6.61 $\log_{10}$ DENV $TCID_{50}$/ml (corresponding to 8.42 $\log_{10}$ Geq/ml) and > 5.81 $\log_{10}$ CHIKV $TCID_{50}$/ml (corresponding to 10.49 $\log_{10}$ Geq/ml) to the limit of detection in RBCs. In all experiments, no infectious viruses were detected after completion of the inactivation process.
Summary / Conclusions: These new data combined with previous published studies show that the photochemical process using S-59/UVA inactivates ZIKV, DENV and CHIKV to the limit of detection in plasma and platelets, and the chemical process using S-303/GSH inactivates these three pathogens to the limit of detection in RBCs. These studies demonstrate a complete solution for the robust inactivation of ZIKV, DENV, and CHIKV in all blood components. As PI has broad spectrum effectiveness against bacteria, viruses and parasites, the technology is of particular interest in arbovirus endemic regions that may be impacted with other transfusion-transmitted infectious diseases.

4C-S28-05

AN ALTERNATIVE METHOD FOR QUALITY CONTROL USAGE IN INFECTIOUS DISEASES TESTING

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Background: The use of external run control (EQC) samples to monitor the performance of assays used to screen for blood-borne pathogens is highly recommended. Traditionally, the mean $\pm$ two standard deviations of the first 20 EQC test results are

used to establish the acceptance range for a particular EQC lot, and Westgard rules to identify failures for EQC testing results. However, no systematic review of the applicability of this approach for infectious disease serology testing has been reported. The majority of users of the 'traditional approach' (designed mainly for clinical chemistry) have experienced difficulty in its application to immunoassay based testing such as serology testing used for blood donor screening. This study compares the frequency of failures of EQC results using Westgard rules with NRL's QConnect Limits when applied to commonly used donor screening assays.
Aims: To determine the frequency of EQC failures using two different monitoring methods in a blood donor population
Methods: Participating laboratories tested a single lot of each EQC sample, QConnect Purple and QConnect HIVp24, in the Abbott PRISM HIV Ag/Ab Combo ChLIA and QConnect Purple in the Abbott PRISM HTLV-I/HTLV-II ChLIA and the HBsAg ChLIA and entered results into an internet-based QC monitoring program (EDCNet, www.nrl.gov.au/qconnect). EQC results and associated data for 2015 were extracted and the results subjected independently to six Westgard Rules and QConnect Limits. The number and percentage failure rates were tabulated and compared. A failure rate of greater than 25% of valid test results (results that had passed assay validation criteria) in any of the six Westgard rules or QConnect Limits was considered to indicate that that rule was not fit for the purpose of monitoring EQC results for that assay.
Results: Results were extracted from three blood screening laboratories using a total of eight Abbott PRISM instruments (16 sub-channels) testing for four analytes, anti-HTLV, anti-HIV 1/2, HBsAg and HIV p24 antigen. The number of EQC results for each laboratory/instrument /sub-channel/analyte dataset varied, with anti-HTLV ranging from 142 to 221, anti-HIV 1/2 141 to 800, HBsAg from 142 to 232 and HIV p24 from 190 to 977. A total of 44 of the 64 datasets (68.8%) had more than one Westgard rule fail at a rate greater than 25%. All of the 16 datasets for HIVp24 had one or more rule fail more than 25% of the time, with 10 of 16 (62.5%) having four or more rules with a failure rate of greater than 25%. The highest failure rate for any one rule was 83.9% for a HIV-1 p24 EQC. In contrast 4 of 64 datasets (6.2%) failed according to QConnect limits greater than 25% of the time.
Summary / Conclusions: The monitoring of the results from well-designed EQC provides objective evidence of test systems being in control or highlights unacceptable levels of variation. The methods used to determine variation need to be sensitive to unacceptable change while allowing normal variation. Westgard rules flag an unacceptable rate of rejections and therefore are not fit for the purpose of monitoring the variation in infectious disease serology tests in a blood donor population. The QConnect Limits were found to be more appropriate for this purpose. This concept is a fundamental shift in the approach to monitor EQC results. It provides laboratories testing for infectious diseases using serology tests with an answer to the question 'how much variation is acceptable', which the traditional approach using Westgard rules has not been able to reliably provide to date.

Cellular Therapies: ATMP

4C-S29-01
HOW TO CULTURE RED BLOOD CELLS FOR TRANSFUSION
A Toye

No Abstract available

4C-S29-02
MESENCHYMAL STEM CELLS FOR THERAPEUTIC PURPOSES: POSSIBLE HURDLES
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Background: Mesenchymal stem cells (MSCs) are pluripotent cells, residing in postnatal organ and tissues, which can differentiate into bone, cartilage and fat. Owing to their appealing properties, including their ability to support hematopoiesis and tissue homeostasis, MSCs have been intensively studied for possible therapeutic applications. Studies on MSCs were initially focused on their potential use for tissue regeneration. The observed MSC immunomodulatory functions have then provided the basis for further possible therapeutic applications. Thus, the number of clinical trials evaluating the use of MSCs for the treatment of different diseases has rapidly increased. However, many questions on MSC biology were left unanswered and, probably, this poor understanding of MSC functions accounts for the obtained contradictory results. A hurdle in MSC research is the lack of specific surface antigens for the isolation of a pure clonal MSC population. Although the use of non-clonal populations will probably be inevitable, because of the low efficiency of clonal MSCs to produce daughter cells, new criteria should be set for the reduction of donor heterogeneity, for the evaluation of stem/progenitor cell percentage, and for culturing and *ex vivo* expansion. A crucial issue that should be addressed is the occurrence of senescence in MSCs, which might impair their long-term therapeutic effectiveness and impact on their interactions with other cells. On the other hand, senescence can have an important anti-cancer role, by arresting the uncontrolled growth of transformed stem cells.
Aims: We aimed to analyze possible hurdles for the use of MSCs for therapeutic purposes. In particular, we focused on senescence evaluation, by characterizing the secretome of senescent MSCs and studying its effects on both non-senescent MSCs and cancer cells. Moreover, we studied the role of crucial senescence regulators, such as the retinoblastoma (RB) family of oncosuppressors, in MSC ability to enter senescence.
Methods: The effects of conditioned medium (CM) from senescent MSCs were evaluated on both young MSCs and myeloma cells. Proteomics approaches were used to analyze the MSC secretoma. Silencing of *RB* family members was used to study the RB role in MSC senescence.
Results: We observed that CM from senescent MSCs induced senescence in young MSCs. Through the characterization of senescent MSC secretome, key factors for senescence induction were identified, including insulin-like growth factor binding proteins 4 and 7 (IGFBP4 and IGFBP7). CM from senescent MSCs was also able to induce senescence or apoptosis in myeloma cells. However, secretoma from senescent MSCs previously incubated with myeloma cells, lost its anti-tumor activity, thus pointing to the importance of better understanding the reciprocal effects of MSCs and cancer cells for a safe use of MSCs in patients that might suffer from cancer. We also observed that *RB* family silencing profoundly affected MSC senescence, thus providing a further characterization of this process in MSCs.
Conclusions: Our results pave the way to further investigations aimed to better understand the senescence process in stem cells and to modify the current *ex vivo* MSC expansion protocols, in order to prevent negative senescence-related effects, while preserving its putative anti-cancer role.

4C-S29-03
TISSUE FACTOR EXPRESSION AND HEMOCOMPATIBILITY OF STROMAL CELLS DEPENDS ON ORGAN ORIGIN AND CULTURE CONDITIONS
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Background: Mesenchymal stem/progenitor cells (MSPC) are promising candidates in the field of regenerative medicine and for the therapy of numerous diseases. Optimal cell source and expansion protocols using fetal bovine serum (FBS) or human platelet lysate (HPL) are still under debate. However, after systemic application long term engraftment is low or absent in MSPC recipients. It was recently shown that during systemic application MSPC hemocompatibility depends on intrinsic coagulation-activating properties. An instant blood-mediated inflammatory reaction (IBMIR) can rapidly activate complement and coagulation cascades thus resulting in hyperacute cell clearance. IBMIR has been observed in allogeneic and autologous cell therapy settings and was related to tissue factor (TF) expression on the cell surface.
Aims: In our study we compared TF surface expression and hemocompatibility of MSPC derived from bone marrow (BM), white adipose tissue (WAT) and umbilical cord (UC). We hypothesized that TF may be used as a surrogate safety marker prior to clinical application.
Methods: MSPC were cultured in media supplemented with pooled HPL (pHPL) or FBS. Proliferation, clonogenicity, trilineage capacity and characteristic MSPC surface

markers were analyzed. TF expression levels were tested by quantitative real-time polymerase chain reaction (qRT-PCR), flow cytometry and immunocytochemistry. Pro-coagulant activity of MSPC was analyzed by rotational thromboelastometry with endothelial and mononuclear cells as negative controls. Clotting time (CT) and maximum clot firmness (MCF) of $2x10^5$, $5x10^5$ and $1x10^6$ MSPC from different sources were tested. The specific impact of TF was confirmed by in vitro clotting of AB plasma to factor VII deficient plasma after addition of $1x10^6$ MSPC from different organ origin, respectively.

Results: MSPC proliferation was significantly increased in pHPL- compared to FBS-media while trilineage potential was maintained. Flow cytometry revealed the common CD14-/19-/34-/45-/MHCII- and CD73 + /90 + /105 + phenotype whereas TF expression differed depending on cell source and culture conditions. UC-MSPC in pHPL showed the highest expression of TF (median 99%; range 87–99), compared to UC-MSPC in FBS (59%; 31–78). In contrast, TF expression of WAT-MSPC in FBS was higher (80%; 71–97) than in pHPL (56%; 47–72). Significantly lower TF expression was only observed in BM-MSPC independent of media supplements (<7%). These distinct expression levels were confirmed by immunocytochemistry and qRT-PCR. In thromboelastometry increasing cell amounts of BM-MSPC and UC-MSPC shortened the CT significantly whereas MCF significantly increased. UC-MSPC/pHPL showed the shortest CT (58sec, 51–76) followed by WAT-MSPC/pHPL (105 s, 74–171). In FBS, WAT-MSPC (83sec, 53–113) displayed more pro-coagulant activity than UC-MSPC (91 s, 67–126). BM-MSPC activated coagulation just weakly (pHPL 412 s, 247–492; FBS 345 s, 159–489). All MSPC showed an elongated CT in factor VII deficient Plasma, independent of source and culture conditions.

Summary / Conclusions: We detected high variations of in vitro hemocompatibility of MSPC of different sources and culture conditions. BM-MSPC had the lowest TF expression and the weakest pro-coagulant activity presumably favoring BM-MSPC for intravenous therapy. Due to our results we recommend TF expression analysis for surrogate safety testing or, alternatively, anticoagulant treatment prior to MSPC Infusion.

4C-S29-04

HEPARIN CHANGES THE GENE EXPRESSION PROFILE OF MESENCHYMAL STEM/PROGENITOR CELLS

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Background: Pooled human platelet lysate (pHPL) has been proven to be a promising human alternative for animal sera for in vitro cell culture intended to be used for clinical applications. pHPL contains abundant growth factors and cytokines efficiently boosting cell proliferation in vitro. To prevent pHPL- media from clotting, the addition of porcine heparin is common. We have therefore recently developed a protocol for the mechanical depletion of fibrinogen to avoid heparin in the culture of human mesenchymal stem/progenitor cells (MSPCs) (J Transl Med. 2015; 13: 354). Even though porcine heparin is safely used in the clinic for a century by now, its impact on MSPC biology remains elusive.

Aims: We investigated whether potential heparin effects on gene expression of MSPCs (derived from umbilical cord (UC), white adipose tissue (WAT) and bone marrow (BM)) are global or tissue-dependent.

Methods: UC-, WAT- and BM-MSPCs (each n = 3) were isolated and cultured using three different alpha-MEM medium supplements: (1) 10% pHPL plus heparin, (2) 10% fibrinogen-depleted pHPL or (3) 10% fibrinogen-depleted pHPL plus heparin. Immunophenotyping and differentiation assays were done for all MSPCs isolated. In addition, proliferation and colony forming units (CFU) assays were conducted for four passages. All MSPCs were subjected to whole genome expression analysis (Affymetrix Human Gene 2.1 ST array). Data were analyzed using R/Bioconductor and Panther, KEGG and Reactome analysis tools and confirmative qRT-PCR was conducted.

Results: Independent of MSPC origin and medium composition, flow cytometric analysis revealed a characteristic MSPC phenotype profile (CD73 + /90 + /105 + and CD14-/19-/34-/45-/HLA-DR-) and a comparable osteogenic, adipogenic and chondrogenic differentiation potential. For WAT-MSPCs, there were no significant differences of cell proliferation and clonogenicity in all different pHPL media. Proliferative and clonogenic capacity of UC- and BM-derived MSPCs however, was increased in the absence of fibrinogen and presence of heparin in early passages (p1-p2). Whole genome expression profiling revealed distinct and source-dependent sets of genes being differentially regulated by heparin. Functional enrichment analysis identified heparin-induced regulation of signaling cascades such as the Notch pathway in WAT-MSPCs only. This links the Notch signaling pathway and heparin, both of which are supposed to be involved in maintaining the stemness of progenitor cells. Depending on the source of MSPCs, further signaling cascades were found to be regulated by heparin: integrin signaling, cytoskeletal regulation by Rho-GTPase, cadherin signaling, but also Wnt-, p53-, EGF-, FGF- and PDGF-pathways.

Summary / Conclusions: Here we demonstrate that the influence of heparin depends on the source of MSPCs: WAT-MSPCs' proliferation and clonogenicity were not altered by different pHPL-medium conditions, whereas BM- and UC-derived MSPCs responded to the mitogenic stimulus of heparin containing modifications. Trilineage differentiation potential was shown to be independent of MSPC origin and medium type used. Our gene expression data show that heparin regulates distinct sets of genes and signaling pathways depending on the source of MSPCs. Further analysis is needed to confirm and characterize the influence of heparin on the cell signaling cascades of MSPCs.

Management and Organisation: Abstracts selected for oral presentation

4C-S30-01

BLOOD DONOR DISCLOSURE – RATES OF FILLED ANTIBIOTIC PRESCRIPTIONS BEFORE 4,978,751 BLOOD DONATIONS IN DENMARK

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Background: Before donating blood, potential donors must meet stringent health criteria. These criteria serve to protect the health for donors and recipients alike. A key element in mitigating the risk of transfusion-related transmission of infectious disease is a trustworthy, full disclosure of the donor's health status (e.g. recent infections) as part of the donor questionnaire. However, the efficacy of the donor health questionnaire to correctly identify donors with risk is incompletely known. In Denmark, donors are deferred if systemic antibiotics have been ingested in the 28-day period before donation (28DbD).

Aims: To assess donor disclosure with filled prescriptions as a proxy for antibiotic use in a large national database of blood donors.

Methods: All active blood donors in Denmark from 1998 to 2011 were identified in the Scandinavian Donations and Transfusions database (SCANDAT). In the analysis, the donors were followed from 6 months before first donation to 6 months after last donation. At the individual level, filled prescriptions were identified in the Danish Prescription Register. Predictors of prescriptions 6 months before and after each donation were assessed by multivariable Poisson regression analysis and reported as incidence rate ratios (IRRs) with 95% confidence intervals (CI).

Results: In total, 463,129 donors gave blood 4,978,751 times. The donors were followed for 2,386,962 person-years and they filled 913,850 prescriptions for systemic antibiotics.

Prescription fill rate in the 28DbD period was 8/1000 donations, which was lower than for other months in the interval from 6 months before to 6 months after donation (IRRs: 2.89–5.27 for other months compared with 28DbD). Women filled more prescriptions than men both 28DbD and all other months (28DbD: IRR 1.52, CI: 1.49–1.56; all other months: IRR 1.54, CI: 1.53–1.55). The prescription fill rate in the 28DbD increased with time since last donation (IRR: 1.31 for donations with

>2 years compared with <6 months since last donation, CI:1.27–1.36). Prescription fill rate fluctuated slightly with donor age, calendar year and month.
Donors who filled a prescription for an antibiotic 28DbD also filled more prescriptions in other months (all months combined: IRR 2.06, CI: 2.04–2.08) and were slightly older (mean 44.1 vs 42.3 years) than donors without prescriptions 28DbD. Donors with a prescription 28DbD were at increased risk of a prescription in subsequent 28DbD periods (IR 36/1000 for subsequent donations).
The most prevalent antibiotic prescription was penicillin, which comprised 45% of the prescriptions 28DbD. Analyses of only penicillin revealed similar results.
Summary / Conclusions: Using a comprehensive study database, we found that an antibiotic prescription was filled within the 28 days prior to 0.8% of all donations. Filled prescriptions are only a proxy of bacterial infection or the ingestion of the antibiotic; however, results suggest antibiotic use is prevalent among our donors in proximity to donation. Moreover, donors who once filled a prescription within 28 days of donation were much more likely to do so again. These findings indicate that some donors may have forgotten their use of medication, may not fully understand the donor health questionnaire or are performing self-assessment of risk.

4C-S30-02

LONGITUDINAL CHARACTERIZATION OF PATIENTS RECEIVING BLOOD TRANSFUSIONS IN A 19-YEAR PERIOD: DECLINE IN BLOOD TRANSFUSIONS AS A RESULT OF DEMOGRAPHIC FACTORS

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Background: While the total numbers of blood transfusions are collected nationwide in Germany, the epidemiological characteristics of individuals who receive transfusions have not been well described.
Aims: We were espiacally interested to determine reasons for an observed decline in the number of transfused RBCs.
Methods: In a retrospective analysis we analyzed data from our laboratory information system to characterize patients receiving red blood cell concentrates (RBC) in the University Hospital of Lübeck between 1998 and 2016.
Results: During the 19 year period 88.023 patients received a total number of 441.383 RBCs. The total annual number of RBC units was 23.294 in 1998, reaching 25.221 in 2003. In the years 2007 to 2012 the number of transfused RBC was 23500 plus-minus 500 per year. Since then we observe a yearly declining rate between 3 and 6% reaching 19.598 RBC transfusions in 2016.
While 51.5% of the recipients were male they received 56.2% of the RBCs, this was similar over the whole period. The average age of females receiving RBCs increased by 2.8 years from 65.4 to 68.1, whereas in males the mean rose by 6.6 years from 60.7 to 67.3 years, and the likelihood to receive transfusions increases with age.
The average numbers of RBC per female case decreased from 4.81 units in 1998 to 4.32 units in 2016, whereas in males the numbers of RBCs declined from 6.12 to 5.27, but was stable for the last four years. Although we observed a decline in total numbers of RBC transfusions especially in the years since 2013, the average number per case was similar.
The percentage of transfused RBCs within a surgical department decreased from 63.9% in 1998 to 46.6% in 2016 with a pronounced decline in the years since 2010, whereas the total numbers of RBCs given in nonsurgical Departments were relatively stable over the time.
We therefore compared the number of transfused RBCs in several age groups with population data of Germany and our province Schleswig-Holstein. For the years 2012–2016 we observe a similar slight increase in population data and transfused RBCs the age group 65–69, whereas in the age group 70–74 we observe a dramatic decrease in population as well as in the number of transfused RBCs. This decrease is a result of weak birthrates in the years 1945 to1949 following immediately second world war.
Summary/Conclusions: In summary we observed an increase of age in patients receiving blood transfusions, and a decline in the number of transfusions. Overall surgical departments transfuse less whereas the numbers of transfusions in nonsurgical departments increased. Demographic factors influence the number of transfused RBCs and the demographic gap of the age groups born in 1945–1949 might be an important reason responsible for the declining transfusions of RBCs in Germany.

4C-S30-03

IMPLEMENTATION OF A TEMPERATURE TRACEABILITY SYSTEM FOR THE STORAGE AND TRANSPORT OF PACKED RED BLOOD CELLS (PRBC) FOR SURGERIES AND THE REUSE OF PROPERLY STORED BLOOD PRODUCTS

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Background: In our Hospital, liver and heart surgeries request 5 to 10 cross matched PRBC ready for a possible transfusion during their surgery. We have found that a large percentage of these PRBC were not used and returned to the Transfusion Service. Since the Transfusion Service did not know of its storage condition in the operating room, they were discarded. According to Spanish *Royal Decree 1088/2005, from 16 September BOE num. 225*, PRBC must be kept between 1 and 10°C during transport and storage, without being able to re-cool units that have surpassed this temperature. Approximately 734 units/year were discarded, 3% of the units in our stock.
Aims: With the aim of reducing the amount of returned and discarded PRBC we decided to implement a controlled temperature chain for these PRBC, measuring their temperature while out of the Transfusion Service. To measure the temperature we used the QTA Tracer System by Tridentify.
Methods: The QTA Tracer System uses wireless transmitters that are attached to each blood bag. The tracer registers temperature variations and stores and processes the information. We analysed the results of the PRBC returned from the Heart and Liver operating rooms back to the Transfusion Service of the University Hospital Cruces, from 1 January 2016 through 31 January 2017
Results: During these 13 months 874 PRBC were returned from the Heart and Liver operating rooms. 695 were recuperated (79.5% of those returned) for future use, with future transfusions resulting in no complications. The temperature measurement every 3 min has allowed us to detect and correct errors in our cold chain, which was reflected in the progressive increase of the recuperated PRBC (from 78% in January 2016 to 92% in January 2017). This method has allowed us to preserve PRBC with specific characteristics reserved for patients with special needs, like washed PRBC for a patient undergoing a liver transplantation with a prior case of anaphylactic shock after a plasma transfusion. Of the returned PRBC, 179 (20.5%) were discarded.
Summary / Conclusions: Implementation of a PRBC temperature traceability system during transport and storage in the operating room has allowed us to:

- Improve the cold chain for delivery of PRBC within the hospital, and its storage in the operating room.
- Recuperate 79.5% of the units returned from the operating room. This has allowed us to recuperate 607 units/year.
- Recuperate PRBC with special characteristics that would have been discarded without a controlled temperature system.
- Reduce the number of discarded PRBC from voluntary donations.

4C-S30-04

COMPUTERIZING BLOOD DONOR AND DONATION INFORMATION IN GHANA

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Background: The Southern Area Blood Centre (SABC) of the National Blood Service Ghana (NBSG) is in the final phase of computerizing blood donor information management using the Blood Safety Information System (BSIS). The BSIS software was developed by a South African-based not-for-profit organization, Jembi Health Systems NPC to manage donor and donation information from blood collection to issue and dispatch. The BSIS was developed as part of a larger US Centers for Disease Controls and Prevention (CDC) funded Blood Safety Strengthening Programme, and supports the African Society for Blood Transfusion (AfSBT) stepwise certification and accreditation process.
Aims: This review documents the experiences, challenges and future of implementing the Blood Safety Information System in Ghana.

Methods: The BSIS software was deployed at the SABC in October 2016 and followed by an Installation Qualification (IQ) and Operational Qualification (OQ), two of a three step user validation process required for the implementation of BSIS. All users were adequately trained to the BSIS and the NBSG reviewed its standard operating procedures, donor clinical record form and other documentations in order to use the BSIS effectively. A setback of finding local vendors to supply the needed hardware (barcode label printers and scanners) and consumables (printer ribbons and labels)after several attempts was resolved with the NBSG procuring pre-printed barcode donation identification number labels from a local printing company and identifying a local vendor to supply the barcode label printers and accessories. This delayed Performance Qualification (PQ) of the software. Nonetheless, the NBSG started the PQ in February 2017 pending the supply of the label printers and accessories. In January 2017, two managers responsible for implementing the BSIS software were trained at a BSIS Implementers Academy organized by Jembi to train Blood Service staff to implement the BSIS software. The NBSG plans to "go live" with the BSIS by the end of March 2017.
Results: A major challenge identified as having the potential to derail the implementation of a sophisticated system like BSIS in a resource-limited country is limited access to requisite hardware and consumables. Meanwhile, another key challenge identified during the final phase of the implementation was a troubling gap between the functionality of the software and its application by users to real time work. Managers of the implementation applied a one-to-one engagement strategy to bridge the gap between the inherent value of the implemented BSIS software and the ability of users to put it to work effectively.
Summary / Conclusions: Sustaining the use of the BSIS in Ghana demands that the NBSG re-thinks of its implementation as an internal marketing job where users are engaged constantly, and establishes local distribution channels for supply and maintenance of requisite hardware and consumables. Capacity building by the BSIS Implementers Academy facilitates the shifting of "ownership" of the BSIS to the NBSG, ensuring sustainability. These strategies will empower the NBSG to continue safeguarding activities of managing donors and improving blood safety.

4C-S30-05

IMPLEMENTATION OF EVIDENCE-BASED AND COORDINATED BLOOD SUPPLY MANAGEMENT SYSTEM: ZIMBABWEAN EXPERIENCE

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Background: The need for sufficient and consistent blood supply is integral to the betterment of healthcare in a country. The National Blood Service Zimbabwe (NBSZ) is solely mandated to provide blood and blood products in Zimbabwe. Due to predominantly youth school-based blood donors (16–20 years, contribute 70% of annual collections), this has created a perennial seasonal challenge in blood supply especially during the year-end festive school holidays. In 2015, NBSZ developed and implemented a multi-layer coordination framework strategy for the blood donor mobilization, collection, and distribution to address these persistent problems. Using monitoring and evaluation performance tracker based on the available daily blood-stocks data for 2016, an analysis of the effectiveness of this strategy was conducted in this study.
Aims: To share how a coordinated framework strategy for blood donor mobilization, collection, and distribution can be developed, implemented, monitored, and evaluated for effectiveness.
Methods: A comprehensive coordination framework strategy was developed which aimed to bring more synchronization in the activities of blood donor mobilization, collection and distribution. A key component of this framework was the introduction of branch coordination teams (BCTs) at NBSZ five branches. These are only attended by operational focal staff directly involved in the actual panels booking, collection, and distribution of blood as the primary level to monitor the day-day status of blood supply management. The BCTs reports monthly using a standard template to the National Coordination Team (NCT) composed of three Executive Managers responsible for these three functional areas. The NCT oversees the BCTs operations and regularly apprise the Coordination Directors and the Executive Management. Using the circulated daily blood bank statements, the NCT developed a blood bank tracker so that it is kept abreast with the dynamics and levels of blood supply at each branch, which is categorized into three levels (Green;>=5-days, Amber;>2-<5-days, Red;<=2-days). Analysis of the overall blood supply status for each category (%) was done for 2016 data. We also analyzed the percentage of blood demand vs supply and expiries.
Results: A total of 64,888 blood units were collected in 2016. The results are based on 1205 daily blood supply status reports. On 89% of days (range, 85%>94%) the NBSZ maintained over 2-days supply stock at any given time (74% for >=5-days, 47%>88%). The bloodstocks were in red (<=2-days) on 11% (6%>15%) of the period under study. The supply rate was 94% (84%>98%) and expiries were at 5.5%.
Summary / Conclusions: The healthy blood bank status on the large part of 2016 and the ability to meet blood demand enabled NBSZ to start reversal of the perennial year-end festive seasonal shortages of blood. This is the first-time in two decades that the Service has managed to initiate systematic process to address this challenge. The coordination framework strategy has proved to be quite useful as it appropriately empowers staff at different levels to make evidence-based decisions and institute appropriate response mechanism to optimize blood supply. This low-cost strategy can be gainfully and equally applied in resource-constrained settings striving to effectively and consistently provide blood supplies.

4C-S30-06

STOP THE WASTE OVER THE FESTIVE SEASON - HAVE WE IMPROVED?

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Background: Minimising unnecessary blood waste is supported through government strategies and Stewardship statement along with National Safety and Quality Health Service Standards. The Blood Matters RBC wastage reduction project was established in 2014 to support health services and pathology providers to meet wastage targets set by Governments.
In the financial year 2015-16, 653,914 RBC units were issued nationally, with 2.9% wasted. Victoria received 180,012 of these units, with 3.2% wasted.
Since 2014 the overall trend of RBC waste in Victoria continues to decrease, however, a peak in waste is evident during the festive season (January/February) due to reduced elective surgical activity. The 'stop the waste' festive campaign was initially launched in October 2015 for the 2015/16 festive season, to inform health services that RBC waste peaks in January/February and to explore strategies to minimise. The campaign was rerun for the 2016/17 festive season, with modifications based on previous evaluations.
Aims: To 'stop the waste', increase awareness of and to reduce red blood cell (RBC) waste over the 2016/17 festive season in Victoria, Australia.
Methods: The 2016/17 festive campaign used a checklist to help key stakeholders identify anticipated variations in practice and demand. An initial email communication was sent in August 2016 in response to feedback received from the 2015 campaign evaluation requesting earlier dissemination. Posters and case studies were circulated to promote discussion with the pathology/blood bank regarding anticipated changes in RBC needs. Private hospital Chief Executives were sent a letter highlighting their role in helping to reduce waste by recognising and reporting anticipated variations in practice over the festive period. The campaign reminded staff to check inventory and monitor practices within their health services, comparing usage and waste patterns from previous years. Email followup was also planned to advise/acknowledge progress as results became available.
Results: Overall Victorian RBC waste continues to decrease, from 5.6% in 2014 to 1.8% in Jan 2017. The 2016/17 RBC issues and festive peak is greatly reduced compared to previous years indicating there has been a positive impact to the campaign.

- The six largest users of RBCs reduced RBC orders by 30% in January 2017 compared to December 2016.
- Waste for December 2016 was 300 units (2.1%), compared to 522 (3.5%) in December 2015.
- Waste for January 2017 was 261 units (1.8%) compared to 575 (4.1%) in January 2016.
- Waste for February 2017 was 314 units compared to 524 (3.6%) in February 2016.
- The reduction in red cell wastage over this festive season gave a cost saving of approximately $299,000.

Summary / Conclusions: Increased awareness and communication of the 'stop the waste' festive campaign has resulted in significant RBC waste reduction. The 2016/17 'stop the waste' festive campaign has been a success and could not have been achieved without the engagement and support of the key stakeholders such as the

blood transfusion committees, pathology staff, private hospital CEOs and transfusion nurses/trainers.

Parallel sessions

Immunobiology of blood cells: Immunity against transfused red cells

5A-S31-01

RED BLOOD CELL ALLOIMMUNIZATION: INDUCTION OF IMMUNITY AND POTENTIAL MITIGATION STRATEGIES

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Millions of RBC units are transfused throughout the world annually. Despite the fact that each RBC unit contains multiple foreign donor antigens, only a minority of transfusion recipients will develop detectable RBC alloantibodies. Some factors that influence humoral immune responsiveness to transfused RBCs are obvious, including the necessity of being exposed to a foreign antigen that the immune system can process and present. Mathematically, however, every RBC transfusion meets those criteria. Thus, it remains unclear why some patients form alloantibodies ("responders") while others may be transfused hundreds of times without becoming alloimmunized ("non-responders"). Studies in humans have found an association with CD4 + T-cell markers and responsiveness to transfused RBCs, and have also identified acute or chronic inflammatory disease states as risk factors for alloantibody development. Studies in animal models, which allow single variables to be isolated, have led to an understanding of the critical role played by dendritic cells in transfusion-associated RBC alloimmunization. Murine studies have further established that non-responsiveness to transfused RBCs equates to antigen specific tolerance in some settings: alloantibodies develop to RBCs expressing some blood group antigens only when the initial RBC exposure occurs in close association with a danger signal. Additional studies are needed in humans and in animal models to further understand the complex variables that determine when and how a humoral immune response may be initiated against transfused RBC antigens, such that strategies for preventing alloimmunization and its harmful effects in transfusion and pregnancy scenarios can be developed.

5A-S31-02

ANTI-D IMMUNISATION IN PREGNANCY – WHY ARE WOMEN STILL BECOMING IMMUNISED?

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Background: Despite the recommended use of anti-D immunoglobulin (Ig) prophylaxis, postnatally, antenatally after potentially sensitising events (PSE) and, more recently, routine antenatal anti-D Ig prophylaxis(RAADP) women are still becoming sensitised. Some of these cases may be due to missed or late administration of anti-D Ig (1182 in the latest 5 years of SHOT reporting) but there are concerns that current recommendations may not be adequate.
Aims: To understand more about this, in 2012 SHOT initiated a new study of women found to have immune anti-D detected for the first time in their current pregnancy
Methods: Reporters provide data on booking weight, management of sensitising events in pregnancy, RAADP both in current and previous pregnancy if applicable.
Results: To the end of December 2016 a total of 157 cases had been reported, 42 in women with no previous pregnancies (NPP) and 115 with previous pregnancies (PP). NPP: in 4 cases immune anti-D was detected before 28 weeks, 11 between 28 weeks and before delivery, 25 at delivery, and 6 months post delivery in one woman who had experienced a large, correctly managed, feto-maternal haemorrhage. In one case the time of detection was unknown. Appropriate RAADP was given in 34/42 but was missed in 5/42. Most, 30/42 (71.4%) had no reported potentially sensitising events (PSE) in pregnancy. PP: 102/115 women had carried the previous pregnancy to term. In 50 out of 115 cases (43%) immune anti-D was detected at booking in the index pregnancy, suggesting sensitisation from the preceding pregnancy. Where information was given on the preceding pregnancy, 14/61 (23%) were obese, i.e. >80 kg at booking, and 16/83 (19%) did not receive RAADP. A total of 28 PSE have been reported in the preceding pregnancies of which 19 (68%) were correctly managed In 9 out of 58 (16%) cases where this information was provided, the preceding pregnancy lasted beyond 40 weeks. Postpartum prophylaxis in the preceding pregnancy was correctly administered (including Kleihauer testing) in 62/102, no information in 27 and not given or wrong dose given in 8. Notably, 18/50(36%) of women who were immunised at booking had received apparently 'ideal care' in the preceding pregnancy.
Summary / Conclusions: Women may become sensitised to the D antigen despite apparently ideal management. These findings raise important questions: should obese women receive modified RAADP? Should extra doses of anti-D Ig be given to women whose pregnancy extends beyond 40 weeks? Is anti-D Ig prophylaxis indicated for medical termination without instrumentation? Do twin pregnancies pose a higher risk of antenatal alloimmunisation? However, errors in administration continue to occur so there is clearly a need for ongoing education and training of midwives and laboratory staff. For example, all laboratories issuing anti-D Ig should have a mechanism to follow up issued doses to ensure they have been given and have full traceability recorded.

5A-S31-03

RH-IMMUNOPROPHYLAXIS AND ABO INCOMPATIBILITY PROTECT AGAINST NON-RHD ALLOIMMUNIZATION BY PREGNANCY

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Background: The mechanism through which anti-D prophylaxis (anti-D-Ig) prevents RhD immunization in pregnancy/delivery remains unclear; both epitope-specific and non-epitope-specific mechanisms are proposed. The latter mechanisms seem comparable to the protective effect that has been shown for ABO incompatibility on the development of RhD antibodies before the introduction of Rh-immunoprophylaxis. If such mechanisms underlie the effect of anti-D-Ig, these would also have a protective effect against the development of other antibodies than RhD.
Aims: This study addresses whether anti-D prophylaxis and ABO incompatibility prevent the development of non-RhD irregular erythrocyte antibodies (IEA), to further understand the working mechanisms of anti-D-Ig.
Methods: A case-control study was performed, including Dutch parae-I with only non-RhD IEA, immunized during their first pregnancy/delivery. The proportions of cases having received anti-D-Ig, respectively with a possible ABO-incompatible previous pregnancy (based on blood group of mother, father and second child) were compared to the calculated proportions in the general Dutch population. The protective effect of anti-D-Ig was investigated in all cases with non-RhD IEA, as well as in subgroups with different antibody specificities. In the subgroup analysis of cases with RhE antibodies, the calculated population proportion of women who received anti-D in their first pregnancy/delivery was adjusted for the higher probability of RhE negative women to be RhD negative. Proportions were compared using the Wilson score, a P-value<0.05 was considered significant.
Results: 232 cases were included, of which 1.7% received anti-D-Ig in their previous pregnancy/delivery, compared to 10.2% in the general population (95% CI 0.67–4.35, $P < 0.001$). Furthermore, a significant difference was found in several subgroups: all Rh-IEA, all non-Rh-IEA, RhE-IEA, Kidd-IEA and other IEA.
12.03% of the cases had a possible ABO-incompatible first pregnancy, compared to 19.36% in the general population (95% CI 7.41–18.96, $P = 0.040$). Pregnancies in which the maternal blood group was O were most likely to be incompatible, followed by B and A blood groups.
Summary / Conclusions: Both Anti-D-Ig and ABO-incompatibility seem to have a protective effect against pregnancy-induced non-RhD immunization. Women with coexisting non-RhD-IEA and RhD-IEA were excluded, possibly resulting in less 'high-responders', women very prone to develop IEA, in our study, compared to the population. The proportion of anti-D-Ig administrations in a population, comparable to our study population, was calculated to be between 4.7 and 10.2%. Consequently, it is reasonable to conclude that anti-D-Ig has a preventive effect on pregnancy-induced non-RhD immunization in total and, more specific, immunization with all Rh- and non-Rh-IEA. Based on this report, future research should focus on not

epitope-specific mechanisms of anti-D-Ig, such as RBC clearance and immunosuppression or immune deviation.

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RH GENOTYPE MATCHING: THE EXPERIENCE OF THE AMERICAN RARE DONOR PROGRAM

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Background: The American Rare Donor Program (ARDP) is made up of 89 AABB-accredited immunohematology reference laboratories. Its mission is to locate rare blood for alloimmunized patients. There are multiple *RHCE* alleles that are predicted to encode hr^B- and/or hr^S- phenotypes and there is some evidence that they are not cross-compatible. ARDP accepts requests for RH genotype matched red cell units for patients with *RHCE* variant alleles who have made Rh antibodies such as hr^S, hr^B and Hr^B. Currently, there are more than 1000 donors in the ARDP who carry RH variant alleles including those who are predicted to be hr^B- or hr^S-.
Aims: To examine the requests for RH genotype-matched donor units and compare the *RHCE* alleles in the patients to the RH allele frequency in the donor population of the American Red Cross. In addition, the number of potentially compatible donors was assessed per request with an aim to identify the RH genotypes that are lacking or insufficient in the ARDP cohort.
Methods: Requests for RH genotype matched donors made to the ARDP from April 2015 to August 2016 were reviewed. Patients were required to have the antibody for antigen negative and RH matched requests to be honored. For each patient, their *RHD* and *RHCE* alleles were compiled. The donor lists that were generated at the time of the request were evaluated for the number of potentially compatible donors and their alleles. RH genotype matching used the Punnett Square approach described previously (Keller *et al., Transfusion* 53(2S):174A,2013).
Results: ARDP received 76 requests from 45 patients, 37 had anti-hr^B, three anti-hr^S and four both -hr^B and -Hr^B; also, 10 had other Rh and five non-Rh alloantibodies. Sixty percent of requests were for African American patients with sickle cell disease. The most frequent alleles in the patients were r's (44% had at least one allele, 31% were homozygous), *RHCE*ce733G* (36% carried at least one allele, 20% were homozygous) and *RHCE*ce48C* allele (9% had one allele). The frequency of these alleles in the African American (AA) donor population is 5% (r's), 7% (*RHCE*ce733G*) and 22% (*RHCE*ce48C*) (Keller *et al., Transfusion* 53(2S):28A,2013). Of the 3 requests from patients with anti-hr^S antibodies, there were no compatible donors in the ARDP.
Summary/Conclusions: Though the *RHCE*ce733G* allele has been assigned a phenotype of hr^{B+vw}/- by the ISBT Working Party for Red Cell Immunogenetics and Allele Terminology, it was found in 36% of these requests. Though the *RHCE*ce48C* allele has not been assigned a partial e phenotype by the working party, it was found in 22% of RH genotype matching requests. Finally, although the most common hr^B- alleles associated with RH genotype match requests are not uncommon in AA donors, since most centers do not perform RH genotyping of donors routinely, the ability to identify units to fill these requests is challenging. Filling requests for hr^S- units is especially challenging, as this phenotype is much less frequent in AA donors. More RH genotyping by donor centers will be critical to meeting these requests.

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IMMUNIZATION TO RED BLOOD CELL ANTIGENS IN PATIENTS WITH SICKLE CELL DISEASE IN MARTINIQUE ISLAND, FRENCH WEST INDIES

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Background: Over the last 10 years, transfusion of RBCs in sickle cell disease (SCD) patients has increased and is associated with significant reduction in morbidity and mortality by preventing first stroke. However, this chronic transfusion policy can induce alloimmunization with acute/delayed haemolytic transfusion reaction as a major consequence.
Aims: To determine the frequency of RBC alloimmunization in a cohort of sickle cell disease patients when donors and patients are in a homogenous ethnic background, within the Martinique Island (French West Indies, Caribbean Sea).
Methods: Clinical data were provided by the Centre for SCD of the Martinique University Hospital. The immunohaematological data and transfusion records of 351 major SCD patients were obtained from our medical software, including results from the National Immunohaematology Reference Laboratory. RBC units were systematically matched for the Rh (C, E, c, e) and K antigen and crossmatched by indirect antiglobulin test. In case of presence of RBC alloantibodies, the RBC units were selected "antigen negative" for Fy^a, Fy^b, Jk^a, Jk^b, S and s antigens.
Results: Overall, the phenotypic frequencies for erythrocyte systems were found to be equivalent between donors and recipients. The C-E- phenotype was the most common (53% of subjects). The D-C-E- phenotype was present in 7% of individuals. The rare S-s- type was present in 1.8% of patients. The phenotype combination C-E-, K-, Fy(a-) was present in 41.2% of patients, but 23% if Jk(b-) type was added and 15% is both Jk(b-) and S- types were considered (only 0.64% of Caucasians match such a phenotype). 86% of patients were transfused at least once and >60% were multi-transfused. Immunization to RBCs (alloantibodies, autoantibodies alone and combination of alloantibodies and autoantibodies) affected 29% of our cohort, 27.3% if we take into account only those with alloantibodies alone or combined with autoantibodies, 20% if we excluded the "naturally-occurring" anti-Le^a, anti-Le^b, anti-M, anti-Kp^a and anti-Lu^a. Autoantibodies (with or without alloantibodies) were found in 10% of patients and in 34% of alloimmunized patients. 32.3% of alloantibodies were within the Rh system, 42% if we add Rh autoantibodies (anti-C and anti-E being the most frequent). There were 11% of anti-V/VS. Rh genotyping revealed variant alleles in 87% of individuals. In the MNS system, anti-S was the most frequent antibody followed by anti-M.
Summary/Conclusions: While we could expect a much lower immunization rate in Martinique, we found almost the same value than in continental France (29%). As a result, an intra-ethnic polymorphism seems to significantly occur, despite a genetically homogeneous population, notably within the Rh system. For Rh antibodies, altered Rh alleles in both patients and donors likely contributed to Rh alloimmunization. This supports the relevance of a molecular testing laboratory implementation in Martinique, especially to screen for major RHD/RHCE variant alleles. Besides, to further reduce the prevalence of alloimmunization in our SCD patients, we suggest a transfusion protocol based on a systematic match for the Rh (C, E, c, e), K antigens, and S whenever possible, in addition to a systematic crossmatch.

Clinical: Patient Blood Management

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ADDRESSING CHALLENGES IN PEDIATRIC PATIENT BLOOD MANAGEMENT: ONE BABY STEP AT A TIME

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Red blood cell (RBCs), platelet, and plasma transfusion are common life-saving interventions for pediatric populations with a wide scope ranging from intrauterine transfusion, to transfusing preterm infants, to treating critically ill children on extracorporeal membrane oxygenation, to the child/adolescent/young adult transplanted with a solid organ or hematopoietic cell graft.
With the above varied spectrum of indications, Pediatric Transfusion Medicine (PTM) is not just about 'transfusing little adults'. Rather, PTM has evolved into a subspecialty/independent discipline, especially gaining momentum over the past 5–10 years with dedicated pediatricians, neonatologists and transfusion medicine specialists committed to advancing the clinical care as well as research in this field.
Transfusion decisions, indications and doses in neonates and children are different from those of adults, most often due to their unique physiology and pathophysiology, thus adult patient blood management (PBM) standards cannot be uniformly applied. While PBM programs are being well-recognized in adults, they are quite far from being the standard-of-care in the pediatrics. This write-up assesses the key

elements necessary for development of a successful pediatric PBM program, systematically explores various pediatric specific blood conservation strategies, identifies current available supportive literature, and highlights the gaps in evidence and challenges in further research.

Pediatric PBM program initiatives are important initiatives requiring a cooperative effort between pediatric surgery, anesthesia, perfusion, critical care, and transfusion medicine services, but also need key operational support from the hospital administration, clinical leadership, finance and the hospital information technology personnel. These programs also expand the scope for high quality collaborative research.

Key components of pediatric PBM programs include monitoring pediatric blood utilization and assessing adherence to transfusion guidelines. Peri-operative blood management strategies include minimizing blood draws, restricting transfusions, intraoperative cell salvage, acute normovolemic hemodilution, anti-fibrinolytic agents, and using point of care tests to guide transfusion decisions.

There are numerous areas where collaborative efforts could be done to investigate pediatric and neonatal transfusion and support vital pediatric PBM programs to optimize neonatal and pediatric care.

5A-S32-02

UK-WIDE AUDIT OF RED BLOOD CELL TRANSFUSION IN HOSPICES

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Background: Small observational studies have shown that transfusion can improve symptoms in people with advanced cancer. However, receiving unnecessary transfusions can cause harm (risk of transfusion-associated circulatory overload (TACO) and often means the person cannot do anything else on the same day. It was not known whether people receiving palliative care were receiving transfusions appropriately or whether their symptoms could be managed more appropriately with alternative treatments. No previous national audit of practice has been performed.

Aims: To identify whether patients are being: appropriately informed about the risks and benefits of transfusion; appropriately monitored during transfusion; and assessed and treated for haematinic deficiencies prior to transfusion.

Methods: 138 hospices from around the UK participated. We collected data on all adults (aged 18 years or older) that were given a red cell transfusion at participating sites from October 1st to December 31st 2016. Only a single unit during a transfusion episode was audited, with all other units within that episode recorded as additional units.

Results: 82 hospices (59%) provided data on 460 patients who received a transfusion. 90% (413) of patients had cancer, the rest had primary diagnoses of heart failure, respiratory disease, renal failure or other. 53% (244) were male. 65% (299) were inpatients. Median age was 73 years (range 24 to 100). The commonest reason for anaemia was anaemia of chronic disease (38%; 173), followed by active bleeding (24%; 110) and bone marrow failure (20%; 94). The cause was unknown in 10% (46).

Only 71% (327) of patients were made aware of the transfusion risks, benefits and alternatives prior to transfusion. Only 68 (15%) patients were weighed prior to transfusion, 41 were ≤70 kg and 12 of these (18% of all those weighed) were ≤50 kg.

273 patients had haematinics checked prior to transfusion. 10 had ferritin <15 μg/l (only 4 were being treated with iron) and 12 had B12 < 200 pg/ml, with only one patient receiving B12 treatment prior to transfusion.

65% (341) were only being treated with transfusions for their anaemia. 32% (145) had a pre-transfusion haemoglobin ≥80 g/l and 38 of these had a low haemoglobin as the only reason for transfusion (other reasons were: fatigue; breathlessness; patient request; and haematology patient on chronic transfusion).

97% (446) of patients had their observations checked prior to transfusion; 95% (436) at 15 min after the transfusion had started; and 91% (418) at 60 min after the transfusion had started. Only 8% (74) of patients had one unit of red blood cells, the majority had two units (77%; 345). Only 2 patients had a haemoglobin check between units.

Summary / Conclusions: Patients should always be informed of the risks and benefits of transfusion. Haematinic deficiencies should be corrected. Patients should not receive transfusions based on haemoglobin levels alone.

Patients are more likely to be cachectic, and therefore at high risk of TACO, however very few were weighed prior to transfusion (to enable a weight-related transfusion to be performed), and most had at least 2 units. Patients should be appropriately assessed for TACO, and weight-related transfusion protocols should be considered.

5A-S32-03

RECOVERY FROM ANEMIA AFTER HOSPITALIZATION & ASSOCIATED 30-DAY RATES OF RBC TRANSFUSION, RE-HOSPITALIZATION, AND MORTALITY

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Background: Anemia is a known marker of severity of illness and associated with increased morbidity and mortality. While randomized clinical trials of red blood cell (RBC) transfusion and evidence-based RBC transfusion guidelines support tolerance of in-hospital anemia, clinical outcomes related to anemia following hospital discharge have not been well characterized.

Aims: Our objective was to describe 30-day rates of RBC transfusion, re-hospitalization, and mortality of anemic patients following hospital discharge.

Methods: Retrospective study from 2010 to 2014 which included 94,564 hospitalizations of 61,185 patients who were discharged with moderate or severe anemia (hemoglobin levels less than 10 g/dL) from 21 hospitals and had a subsequent hemoglobin level available within one month. Outcomes and measures included hemoglobin levels, RBC transfusion events, re-hospitalization, and mortality within 1 month of hospital discharge were compared in patients with and without a subsequent rise in hemoglobin levels. Data are presented as percentages and medians and interquartile ranges (IQRs). Accordingly, X^2 tests for equal proportion or Wilcoxon rank sum tests were used to test differences.

Results: The majority (81%) of patients had an increase in their hemoglobin level within 1 month from the time of hospital discharge. The median hemoglobin level at hospital discharge was 9.1 g/dl (IQR 8.6–9.6) and 9.2 g/dl (IQR 8.7–9.7) in patients with and without subsequent rise in hemoglobin, respectively ($P < 0.001$). Post-discharge hemoglobin levels were 10.3 g/dl (IQR 9.6–11.0) and 8.6 g/dl (8.0–9.1) in patients with and without a rise in hemoglobin levels, respectively ($P < 0.001$). Compared to those with hemoglobin recovery, patients without recovery had higher 30-day rates of RBC transfusion (40% vs. 11%), re-hospitalization (52% vs. 35%), and mortality (7% vs. 4%) ($P < 0.001$ for all comparisons).

Summary / Conclusions: Post-discharge hemoglobin recovery differentiated 30-day rates of RBC transfusion, re-hospitalization, and mortality. Better monitoring of hemoglobin levels following hospital discharge may provide opportunities to identify persistent anemia and intervene in high risk patients with increased likelihood of subsequent RBC transfusion, re-hospitalization and death.

5A-S32-04

A NOVEL PATIENT BLOOD MANAGEMENT APP TO GUIDE JUNIOR DOCTOR DECISIONS ON RED CELL TRANSFUSIONS

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Background: Successive audits have shown inappropriate use of 15–30% of blood components. The Patient Blood Management (PBM) patient-centred approach to blood transfusion promotes blood use only when appropriate, substituting alternatives where feasible. Previous UK studies showed that 79% of medical students and 74% of junior doctors owned smartphones with medical related apps.

Aims: 1. To develop a Class I CE marked Smartphone Application (App) to improve junior doctor compliance with transfusion guidelines and reduce inappropriate red cell transfusions in non-bleeding adult patients, emphasising informed patient consent and anaemia management.

2. To develop scenarios with gold standards and use these to test the App's accuracy.

Methods: A multidisciplinary team with junior and senior clinicians, transfusion practitioners, digital healthcare and biomedical scientists developed the App screens and logic.

We developed 30 transfusion scenarios based on medical or surgical cases and sent these to 18 consultant haematologists with expertise in Transfusion Medicine (minimum three consultants per scenario). To obtain a gold standard verdict and identify ambiguous scenarios we compared consultant decisions and decision certainty on whether or not transfusion was indicated using a visual analogue scale for each

scenario. The consensus clinical decision was then compared with the guidance consultants obtained using the App.
Results: Of the 30 scenarios, one was excluded as there was no consultant consensus; four others showed less than 100% consensus. Krippendorf's alpha was 0.71 for the 29 scenarios and Bland Altman plot showed no bias. We examined App performance on 29 scenarios against a 'gold standard' (at least 2 verdicts with the same decision). The App was unable to provide clear guidance on 6 (21%) of 29 scenarios. When two or three consultants independently tested the App on the same scenario (23 scenarios), they obtained identical results in 15 scenarios, i.e. 65% user agreement. This suggests that app data entry screens need improvement (eg. by clarifying data definitions) and / or the advice wording needs to be modified.
Using the majority app verdict, the crude accuracy (agreement) rate against the gold standard was 21/23 (91%) with two false positive errors (app suggested transfusion, gold standard was not to transfuse), a sensitivity of 100% and specificity of 85%. The app suggested that patients should be transfused in 12 (52%) scenarios and was correct (PPV) 83% of the time; when it stated that patients should not be transfused (NPV) it was 100% correct.
Summary / Conclusions: Based on this evaluation, the Medicines Healthcare Products Regulatory Agency in the UK have approved CE marking of the App. We will now undertake further assessments of these scenarios with junior doctors, comparing decision making before and after use of the App followed by a field trial to assess App impact on junior doctor clinical decision making with measuring outcomes around key aspects of PBM eg. appropriate anaemia management, obtaining informed patient consent and use of single unit transfusions. Consideration is also being given to the scenarios that highlight areas where consensus on transfusion was low, even amongst lead Transfusion Consultants.

5A-S32-05

ASSOCIATIONS BETWEEN PREOPERATIVE ANAEMIA, TRANSFUSION AND OUTCOMES IN OLDER HIP FRACTURE PATIENTS

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Background: Anaemia in hip fracture patients has been associated with increased risk of allogenic blood transfusion (ABT), poorer functional outcomes and increased mortality. While incidence of anaemia after hip fracture surgery has been reported to be as high as 86%, few studies have reported the prevalence of anaemia on admission or it's progression prior to surgery in the older hip fracture population.
Aims: To measure the prevalence of anaemia on admission in older persons who sustain a hip fracture, identify if anaemia progresses prior to surgery and report associations with ABT, length of stay (LOS) and in hospital mortality (IHM).
Methods: A retrospective, observational study was undertaken in a regional hospital. All patients aged 60 and over, admitted with a primary hip fracture resulting from a simple fall, in the 12 months of 2014 were included. The world health organization (WHO) definition of anaemia was used. Pathology databases and clinical records were reviewed to collect the following data for the period between admission and surgery: demographics (age and sex), fracture type (intracapsular or extracapsular), use of anticoagulant or antiplatelet medication (excluding aspirin), pre-surgery serial haemaglobin (Hb) levels (as available), LOS, packed red blood cells (PBRC) transfusions during the admission, and IHM. Repeated measures ANOVA's were used to quantify the progression of anaemia prior to surgery, and Chi square and Fisher's exact tests were used to report associations with outcome variables.
Results: A total of 261 patients were identified. The median (IQR) age was 84 years (11). There were twice as many females (n = 186) as males (n = 75) and just over half the sample had extracapsular fractures (n = 141). Anaemia was present on admission in 45% (n = 117),with mean Hb (SD) 122.9 g/L (16.5). The highest incidence of anaemia occurred in males 51% (n = 38), extracapsular fractures 53.8% (n = 77) and those aged over 80 years 52% (n = 95). Progression of anaemia prior to surgery was significant in all groups ($P < 0.05$). Mean time from admission to surgery was 1.23 days. The mean Hb decrease prior to surgery was 13 g/L with the greatest reduction (19 g/L) seen in extracapsular fractures. Pre-surgery reduction in Hb was recorded in 82.9% of patients between admission and day 1, and in 69.8% between day 1 and day 2. Overall the postoperative transfusion rate was 29.1% with the highest rate in extracapsular fractures (43.6%). There was significant association between anaemia on admission and PRBC transfusion ($P < 0.05$) and hospital mortality ($P < 0.05$). There was no association with the use of antiplatelet or anticoagulant medication, nor LOS.
Summary/Conclusions: The findings demonstrate that pre surgical anaemia in older hip fracture patients is associated with a PRBC transfusion and increased hospital mortality. Importantly, it also identified that patients continue to bleed after admission, leading to the development of or worsening anaemia. Thus identification anaemia in the pre surgical period provides an opportunity for treatment to avoid transfusions and improve patient outcomes.

Blood Safety: Impacts on testing

5A-S33-01

DRIVERS OF INFECTIOUS DISEASE THREAT EVENTS: IMPLICATIONS FOR THE BLOOD SUPPLY

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The introduction and dispersal of tropical pathogens to Europe and its neighbouring countries commonly associated with warmer temperatures pose a threat to the supply of substances of human origin, particularly if they are unknown or without diagnostic tests. Emerging infectious diseases (EID) continue to be a threat to Europe and the increase in EIDs over the last five decades continue to pose a threat to substances of human origin. Thus, the emergence of infectious diseases has highlighted the importance of understanding the underlying drivers of EIDs. By recognizing and disaggregating multiple drivers, the process of emergence can be disentangled, and the drivers can then be categorized and prioritized for public health action. For example, it might be possible to anticipate and mitigate the impact of EIDs, through monitoring and surveillance of such drivers.
Infectious disease threat events (IDTE) registered through epidemic intelligence at the European Centre for Disease Prevention and Control (ECDC) that occurred between 2008 and 2013 were collected from the ECDC's event-based data registry. Epidemiological information related to the underlying drivers of these IDTEs was reviewed from a number of sources (e.g. peer-reviewed literature, rapid risk assessments of outbreaks). Drivers were categorized into three main groups: globalization and environment, social and demographic, and public health systems.
Observed drivers for each IDTE were sorted into three main groups: globalization and environmental drivers contributed to 61% of all IDTEs, public health system drivers contributed to 21%, and social and demographic drivers to 18%. Multiple regression analyses of the most frequently occurring observed IDTEs in Europe showed that four of the top five drivers were in the globalization and environment group: travel and tourism, natural environment, global trade, and climate.
Monitoring and modelling such disease drivers can help anticipate future IDTEs and strengthen control measures. More important, intervening directly on these underlying drivers can diminish the likelihood of the occurrence of an IDTE and reduce the threat to substances of human origin. For example, monitoring the distribution of relevant vectors and of areas affected by an outbreak; defining the geographical areas where safety measures need to be considered; ensuring preparations of national/regional risk assessment of transmission; defining appropriate safety measures in the national preparedness plan; etc.
In the interest of protecting the integrity of substances of human origin, it is desirable to detect IDTEs early in order to enable a rapid public health response. Analysis of the drivers of IDTEs in Europe can strengthen vigilance against epidemic threats and safeguard substances of human origin.

5A-S33-02

PREVALENCE AND INCIDENCE OF HUMAN T-LYMPHOTROPIC VIRUS TYPES 1 AND 2 INFECTIONS IN UNITED STATES BLOOD DONORS, 2008–2015: IS A SELECTIVE TESTING STRATEGY JUSTIFIABLE?

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Background: All United States (US) blood donors are tested at every donation for antibodies to Human T-Lymphotropic Virus (HTLV) Types 1 and 2. Because the rate of new HTLV infections in donors is believed to be extremely low, blood services in some countries have discontinued universal testing and implemented a selective testing strategy. To assess the appropriateness of one-time, selective testing for HTLV in

US donors, we evaluated four 2-year incidence periods for HTLV seroconversion in repeat donors. Prevalence rates and associated demographics were also examined.
Aims: Calculate the prevalence and incidence of HTLV confirmed seropositivity in the American Red Cross (ARC) donor population (first-time and repeat donors) from 2008–2015.
Methods: ARC data were used to calculate the overall prevalence of HTLV-1 and HTLV-2 in all allogeneic blood donors and rates stratified by sex, age, race/ethnicity, donor status and US region. Incidence densities for four time periods (2008–2009, 2010–2011, 2012–2013, 2014–2015) were calculated using repeat donors having at least two donations in the 2-year period.
Results: Between 2008 and 2015, more than 47 million donations were tested from over 13 million donors with 1071 HTLV seropositives identified. Overall, HTLV donor prevalence was 8.2 per 100,000 donors. Donor prevalence differed significantly by sex, age, race/ethnicity, donor status and US region. First-time donor prevalence was 12.2 per 100,000 first-time donors compared to 2.1 per 100,000 in repeat donors. There were 781 positive female donors (11.6/100,000) and 290 male donors (4.5/100,000). The highest prevalence was seen in African-American donors (50.9/100,000) and the lowest in Caucasian donors (2.7/100,000). During 8 years of observation there were 108 repeat positive donors, but only 37 were considered to be incident donors with a prior non-reactive donation within 2 years of their positive donation (71 were censored). Female donors accounted for most incident donors (32 vs. 5 males). The majority were typed as HTLV-1 (n = 21) with 11 HTLV undetermined and 5 HTLV-2. Based on the 13 seroconverting, repeat donors in 2008 and 2009, an overall incidence density (ID) of 0.30 per 100,000 person-years (py) was calculated (95% CI, 0.16, 0.52). In 2010 and 2011, there were eight incident donors with an overall ID of 0.19 (95% CI, 0.08, 0.37). In 2012 and 2013, there were eight incident donors with an overall ID of 0.20 (95% CI, 0.09, 0.41). In 2014 and 2015, there were eight incident donors with an overall ID of 0.23 (95% CI, 0.10, 0.45).
Summary/Conclusions: Differences in HTLV donor prevalence rates for years 2008-2015 by donor characteristics were observed. Prevalence was highest in first-time donors, female donors, donors 50–59 and 60–69 years old, African-American donors and donors from the West and South US. Since 37 incident infections were identified in repeat donors (0.19–0.30/100,000 pys for the 2-year periods observed), one-time screening for HTLV antibodies would allow newly infected donors into the blood supply and potentially compromise recipient safety.

5A-S33-03

EVIDENCE OF THE COVERT RISK: PROPIONIBACTERIUM ACNES, A PREDOMINANT RED BLOOD CELL CONTAMINANT, INVOLVED IN A SEPTIC TRANSFUSION REACTION

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Background: The anaerobic skin flora bacterium *Propionibacterium acnes* is a common contaminant of red blood cell (RBC) concentrates. Remarkably, not many cases of transfusion reactions associated with *P. acnes* have been reported. This case report investigated a recent transfusion event involving a 59-year old male patient suffering from acute leukemia who was transfused with a 40-day old RBC unit. Thirty minutes after stopping the transfusion, the patient developed fever, decreased blood pressure, hypoxemia, and confusion. The patient was successfully treated with analgesics, diuretics, antibiotics, and supplemental O_2. Bacterial cultures from the RBC unit and the patient were positive for *P. acnes*.
Aims: 1) To determine the prevalence of *P. acnes* as a RBC contaminant at Canadian Blood Services using Quality Control (QC) sterility data. 2) To investigate whether the isolates of *P. acnes* obtained from the RBC unit and the patient involved in the transfusion reaction are clonally related.
Methods: QC testing of outdated (43-day old) RBCs is performed monthly for 1% (or a minimum 10) units using aerobic and anaerobic BacT/ALERT culture bottles. QC data obtained from 2013 to 2016 were analyzed to determine the percentage of contaminated RBC units and the identity of the bacterial isolates. To further the investigation of the transfusion septic case, the identity of the isolates obtained from the RBC unit and the patient was confirmed using Gram staining and biochemical profiling with the Analytical Profile Index (API). The isolates were further characterized by the PCR amplification and sequencing of the housekeeping *recA* gene. Furthermore, genotyping was performed by repetitive extragenic palindromic (REP) - PCR fingerprinting using primers against the BOX element.
Results: QC data showed that 19 out of 34,810 RBC units tested (0.05%) were contaminated with bacteria. Isolated species included eight diphtheroid bacilli (not *Corynebacterium diphtheriae)* (42.1%), seven *P. acnes* (36.8%), two *Propionibacterium* spp (10.5%), and two coagulase negative staphylococci (CoNS) (10.5%). While the diphtheroid bacilli and propionibacteria were isolated exclusively from anaerobic culture bottles, the two CoNS were isolated in both aerobic and anaerobic bottles. The patient and RBC bacterial isolates involved in the septic transfusion reaction case were confirmed to have Gram negative short rod morphologies consistent with *P. acnes*, and identical API biochemical profiles. Furthermore analysis of the *recA* gene sequence and REP-PCR fingerprinting demonstrated that both isolates belong to the same phylotype and have identical banding patterns.
Summary/Conclusions: The biochemical profiles taken together with the genetic characterization and fingerprinting confirm that the bacterium isolated from the patient originated from the transfused RBC unit. There are no published reports of transfusion reactions involving RBCs contaminated with *P. acnes*. However, this bacterium has been associated with colonization of biomedical devices causing endocarditis. The QC data demonstrate that skin flora anaerobic bacteria are the predominant RBC contaminants and are able to survive during RBC storage at 1–6°C for more than 42 days. These results reiterate the need for a heightened awareness of the safety risk posed by blood products contaminated with anaerobic organisms and the need to improve hemovigilance measures.

5A-S33-04

INFECTIVITY OF BLOOD PRODUCTS CONTAINING CYTOMEGALOVIRUS DNA: RESULTS OF A LOOK-BACK STUDY IN NON-IMMUNOCOMPROMISED PATIENTS

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Background: DNA of human Cytomegalovirus (CMV) is frequently detected in plasma of donors with primary CMV infection. It is unknown, however, whether leukodepleted blood products from these donors contain sufficient amounts of infectious virus to cause transfusion-transmitted CMV infections (TT-CMV).
Aims: This study aimed to determine the rate of TT-CMV in patients receiving CMV DNA-positive blood products.
Methods: During a 14-year-period, CMV DNA-positive donations were identified as part of several previously published studies. Additionally, further donors with seroconversion were tested for CMV DNA. The serostatus of patients who had received a CMV DNA-positive blood product was determined out of pretransfusion samples. Later samples were examined for development of CMV antibodies. Patients with a follow-up of less than 140 days were also tested for CMV DNA.
Results: 221 blood products from CMV DNA-positive donations were transfused to 219 recipients. Pretransfusion samples were available for 179 patients, of whom 62 (34.6%) were seronegative. For 39 seronegative recipients of 40 blood products follow-up-samples drawn at least 30 days after transfusion were available. The median duration of follow-up was 287 days (range 38–3784 days). 36 patients were still CMV-seronegative in their last sample. Three patients were CMV-seropositive due to passive antibody transfer by plasma rich products from seropositive donors, but CMV DNA-negative in all tested samples.
Summary/Conclusions: TT-CMV was excluded in all recipients of 40 blood products from CMV DNA-positive donations. This corresponds to a 95% interval of confidence for the risk of TT-CMV of less than 7.4%. Because none patient belonged to a typical at-risk population, the results are only valid for immunocompetent subjects.

5A-S33-05

PRELIMINARY PERFORMANCE CHARACTERISTICS OF A BABESIA NUCLEIC ACID TEST ON A FULLY AUTOMATED SYSTEM, AND RESULTS FROM SCREENING DONATED BLOOD FROM ENDEMIC REGIONS OF THE UNITED STATES

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Background: The Procleix® Babesia assay for use on the Procleix Panther® system is a Transcription-Mediated Amplification (TMA), qualitative *in vitro* nucleic acid test currently under development. The assay is designed to detect four *Babesia* species (*B. microti*, *B. divergens*, *B. ducani*, and *B. venatorum*) in human whole blood specimens. The assay uses an automated whole blood sample lysis preparation method prior to testing on the Panther system. This test is intended to screen blood donations individually and in whole blood lysate pools of up to 16 donations.
Aims: The aims of these studies were to determine the preliminary analytical sensitivity, clinical sensitivity in individual and 16-donor pooled lysates, and specificity of the Procleix Babesia assay on Panther system.

Methods: To determine the preliminary analytical sensitivity, dilutions of *in vitro* synthesized RNA transcripts for the four clinically relevant Babesia species were evaluated and results were subjected to probit analysis (SAS Enterprise Guide 6.1). Fresh Babesia-infected hamster whole blood with a known number of parasites was used to determine the limit of detection (LOD) of parasites/ml. To determine clinical sensitivity and specificity, 27,076 un-linked whole blood donations collected by the American Red Cross from August 25th 2016 to February 24th 2017, mainly in Connecticut, New Jersey, Pennsylvania, Maine, New Hampshire, and Vermont were screened using the Procleix Babesia assay under a research protocol (Research Use Only study). Initially reactive donations were confirmed by repeat testing, PCR, and serology testing. Reactive individual donor lysates were also tested in pools of 16.
Results: The Procleix Babesia assay detected all four Babesia species with a 95% LOD ranging from 7.10–13.51 copies/ml and fresh Babesia-infected hamster blood with a 95% LOD ranging from 1.31–3.61 parasites/ml. Of the 27,076 donations screened, 17 initial reactive and 14 confirmed reactive donations were identified, for a specificity of 99.989% (95% CI: 99.967–99.996%). All confirmed reactive samples were also reactive in pools of 16. From the 14 confirmed reactive specimens, 13 were reactive by IFA and none by PCR. One was detected apparently during the window period prior to seroconversion. Donors of reactive donations resided in Connecticut (11), New Jersey (1), New Hampshire (1) and Maine (1) for an overall incidence of 1:1,934, and an incidence in Connecticut of 1:1,187.
Summary/Conclusions: The Procleix Babesia assay on the Procleix Panther system demonstrated high clinical specificity and sensitivity and detected all four *Babesia* species. All confirmed reactive donations were also detected in pools of 16 thus demonstrating the potential effectiveness of pooled lysate screening.

Resource Limited Countries: Improving Blood Transfusion in the Developing World

5A-S34-01

PRACTICAL EXPERIENCES FROM THE FIELD: REFLECTIONS OF BLOOD SAFETY IN AFRICA

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Background and Objectives: SAFE BLOOD is the foundation stone of Health Systems Strengthening throughout low-resource countries. Failing HSS initiatives can be traced back to inadequate supplies and poor access to tested and crossmatched blood. Most medical procedures assume blood is available. Often national disasters become tragedies without safe blood. The developed world has National Blood Services that supply blood from well-managed donor panels. These are largely non-existent in Africa. The purpose of this paper is to reflect on observations during Technical Assistance activities over seven years.
Materials and Methods: The reflections here are based on observations during Technical Assistance activities developing Blood safety in several African countries. These are supported by reviewing recent literature and commentary on new technologies and global changes potentially impacting Blood Safety and HSS in Africa.
Results: Africa is the reciprocal of the developed world where c. >75% of blood, donated by Voluntary Non-Remunerated Donors, is used for planned surgeries, oncology and trauma (car accidents mostly). Sadly the developing world uses over 70% of its inadequate blood supply (< 50% of needs) to treat obstetric haemorrhage and acute anaemia in children under five suffering from advanced malaria. Those familiar with blood safety practices will assume blood collected is made into the components of red cells, platelets and plasma. The global multi-billion dollar plasma industry pays for their plasma. WHO still advocate voluntary donors are the safest, but some advocate that this 'industry' should be dropped into Africa providing incentives to 'donors', solving the supply problem.
Conclusion: The assumptions of available and assessable safe blood for any clinical practices are mostly failed! There are many possible solutions but cost recovery models must become the norm. As there are few clinical indications for plasma in Africa so those few countries making components discard an estimated $30 Million worth! While whole blood is the norm, given the declining funding for blood safety, a way needs to be found to secure this resource, channelling all profits into improved Blood Safety based on voluntary donation. New technologies could help.

5A-S34-02

THE ORGANISATION OF BLOOD CENTRES IN NAMIBIA

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In Namibia, blood transfusion activities are centrally coordinated through the National Blood Policy which defines the roles and responsibilities of all stakeholders in the National Blood Program, namely; the Blood Transfusion Service, Government of Namibia (through the National Blood Authority) and transfusing hospitals through their Hospital Transfusion Committees (HTC) and Blood Banks. The Blood Transfusion Service of Namibia (NAMBTS) was incorporated in 1963 as a non-profit organisation and is the only institution licensed by the Government of Namibia to collect, test, and distribute blood and blood products to hospitals in the country. NAMBTS is governed by a Board whose members are blood donors elected to serve by the donors themselves.
NAMBTS collects 35 000 donations from voluntary non-remunerated blood donors annually of which 97% are whole blood and 3% apheresis platelets. Whole blood donation rate is 13.1 per 1000 population and about 70% of all donations made are from repeat donors with a donation frequency of 2.1. All whole blood donations are processed into components and even though all red cell concentrates are transfused, with an out- dating rate of 2%, only 30% of recovered plasma is transfused. Surplus plasma is exported to South Africa for fractionation and the Plasma Derived Medicinal Products are imported back into Namibia and distributed to hospitals.
Blood is tested for Transfusion Transmissible Infections (TTI); Human Immunodeficiency Virus (HIV), Hepatitis B Virus (HBV), Hepatitis C Virus (HCV) and Syphilis using Individual Nucleic Acid Amplification Test (ID-NAT) and serology. Namibia is the second country in sub-Saharan Africa after South Africa to implement ID-NAT for HIV, HBV and HCV on all donated blood thereby interdicting serological window period especially in our setting where the prevalence of HIV and HBV in the general population is high. The total prevalence of TTI markers among donations is 1.7% (HIV-0.2, HBV-0.7, HCV- 0.4 and Syphilis- 0.4).
Blood is supplied to patients through 50 hospitals and 85% of blood is used in government run (state) hospitals. Each hospital has a HTC that oversee adequacy and appropriate clinical use of blood and blood products. Namibia has a national haemovigilance system that covers all aspects of the transfusion chain; from blood collection to the follow up of the recipients, gathering and analysing all adverse effects of blood transfusion in order to correct their cause and prevent recurrence.
NAMBTS operates on a cost recovery basis by billing the recipients of blood whereby the government pays for state patients and health insurance pays for private patients. NAMBTS received PEPFER funding between 2004–2014 which contributed about 14–61% to operating income with the remainder coming from cost recovery but currently the Service is not receiving any donor funding.

5A-S34-03

MOTIVATING BLOOD DONORS IN GHANA

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Introduction: In Ghana, and across sub-Saharan Africa (SSA), blood shortages are common and there is heavy reliance on family/replacement donors (FRD). Although there have been improvements in the last few years, in Ghana in 2015 and 2016 only 62–64% of the annual requirement of 250,000 units were collected and FRD constituted around 65% of donors. Over half of these were first time donors despite recognition that repeat donors are safer. To meet its targets for blood collection and voluntary blood donors (VBD), the Ghana blood service (NBSG) needed locally-relevant evidence about how to better motivate blood donors and promote blood donations.
Aims: To describe the available evidence about factors that influence blood donor motivation and activities that have been undertaken to promote blood donation, and make recommendations about how to improve blood donation in Ghana.
Methods: We reviewed four published papers and three conference abstracts identified from searching PubMed, African Journals Online (AJOL) and ISBT conference abstracts from August 2016 to January 2017, and five unpublished student study reports on blood donor motivation in Ghana. We also reviewed activities undertaken by the NBSG, since its inception in 1973, to promote blood donor motivation.
Results: The evidence indicated that 71–94% of donors had a positive attitude towards blood donation and 95–99% were willing to donate again. Saving lives (78–94%) and reminders (60–90%) were strong motivators for both VBD and FRD. Donating for a family member, being asked to donate, experiencing need for blood, ease of access to donation site, being in good health, gifts and incentives, being thanked and being given information about blood donation were also motivators. Factors that discouraged donation were dislike of needles, ill health, misperceptions about the spirituality of blood and the use of blood for rituals/occultism/evil sacrifice, fear of losing strength/health, and lack of knowledge or information. Activities

undertaken to promote and motivate donation included incentives and blood crediting/group assurance schemes, and pre-depositing blood by families of antenatal women and patients undergoing elective surgery. There were also examples of collaborations with corporate organizations, media, civil societies, social groups, faith based organizations, health facilities, non-governmental organizations and governmental agencies for improving donations. These focused on education, sensitization, recruitment campaigns, blood donation campaigns and drives, community mobilization, peer recruitment, social media engagement, modern communication systems, donor information management and research.

Conclusions: The NBSG has made progress towards improving blood donation rates but analysis of the available evidence has highlighted several measures that could be taken to increase recruitment and retention of altruistic repeat blood donors. It is important that this evidence is now used to influence policies and activities regarding donor recruitment and retention and to make a case for strategically strengthening national blood services.

Plenary Session: Blood donor studies and Big data

PL3-01

THE INTERVAL STUDY: AN RCT OF 50,000 DONORS TO OPTIMISE DONOR HEALTH AND THE BLOOD SUPPLY

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Introduction: One approach to T Interval Study Group manage the blood supply is to understand the relationship between the frequency of blood collection and donor symptoms and safety. Currently, NHS Blood and Transplant recommend donation every 12 weeks (men) and 16 weeks (women) although standards elsewhere in Europe can be as frequent as every 8 weeks. The primary aim of INTERVAL is to determine if donation intervals can be reduced whilst maintaining the health of the donor.

Design and methods: 50,000 male and female donors have been recruited at donation centres in England between June 2012 and May 2014. Donors were randomised to one of three gender-specific donation frequencies for 2 years. The primary outcome was the number of donations per year and the main secondary outcome is quality of life (using Short Form Health Survey SF36v2). Further secondary outcomes are number of low haemoglobin deferrals of donors, iron status, cognitive function and physical activity. Data have been collected via online questionnaires.

We have studied how far the trial participants were representative of the general donor population. All donors recruited into the study met NHSBT haemoglobin (Hb) screening criteria by our gravimetric (copper sulphate) method (males: >135 g/l, females: >125 g/l). As part of the study protocol, full blood counts (FBCs) were performed using a Sysmex XN2000 analyser within 24 h of collection and the genetic traits associated with blood cell phenotypes have been determined.

The characteristics of donors eligible and consenting to participate in INTERVAL have been compared with the general donor population, using the national blood supply database for the period of recruitment. The characteristics of participants recruited from different sources are also compared, as well as those who were randomised vs those not randomised.

Results: The study achieved: integration of research protocols in routine donation practice; good questionnaire response (~80%) and good adherence by participants to allocated donation frequencies. The study was completed in June 2016 and is being analysed. The majority of donors with low Hb, high Hct or low Plt count had normal values upon retesting. However 66% of donors with initially high platelet counts (0.1% of enrolled donors) had repeatedly high counts and were referred to their GP for further investigation. From a total of 542,605 invitations, 48,725 donors were eligible and consented to participate in INTERVAL. The characteristics of participants were similar to the general population of 1.3 million donors in terms of ethnicity, blood group distribution, and recent low haemoglobin deferral rates. However, INTERVAL participants included more men (50% vs. 44%), were slightly older (mean age 43.1 vs. 42.3 years), included fewer new donors (3% vs. 22%), and had given more donations over the previous two years (mean 3.3 vs. 2.2).

Conclusions: INTERVAL is generating scientific evidence on which to base future blood collection policies in England, and potentially elsewhere. It will yield, the currently lacking, reliable data on the effect of donation frequency on blood supply and donors' physical and mental well-being. A full list and contributions of the study group members can be found at www.intervalstudy.org.uk.

PL3-02

BIG DATA IN TRANSFUSION MEDICINE: FROM DONOR AND RECIPIENT HEALTH AND BEYOND

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Background: Each year, millions of individuals worldwide volunteer to donate 112.5 million units of blood to meet the transfusion demands of a similar number of critically ill patients. For public health and other reasons it is therefore pertinent to identify and prevent potential adverse health effects of donation and transfusion. While certain risks associated with donation and transfusion are well established, e.g. iron depletion among blood donors and disease transmission to recipients, it has so far been technically challenging to explore possible late effects of donation and transfusion on a larger scale.

Aims: To study the health of blood donors and transfusion recipients and the interaction between the two by the application of large-scale data. We further want to answer relevant general health research questions using data from blood donors and transfusion recipients.

Methods: The Scandinavian Donation and Transfusion database (SCANDAT2) contains linked data of 25.5 million donations and 21.3 million transfusions in Sweden and Denmark in the period 1968-2012. The Danish Blood Donor Study (DBDS) has since March 2010 enrolled more than 110,000 participants with questionnaire data and a biobank of >500,000 plasma samples. DBDS has since 2015 used tablet computer based questionnaires. Both SCANDAT2 and DBDS have extensive linkage to a large number of relevant national health and socio-demographic registers allowing prospective and retrospective tracking of medical and social data.

Results: Reassuring for transfusion safety SCANDAT studies have demonstrated that conditions such as cancer, Parkinson's disease, and dementia are unlikely to be transmitted with blood transfusion, and have moreover rejected hypotheses of clinical effects of red cell storage, donor age, and donor sex.

Concerning donor health, we have demonstrated a strong selection of healthy individuals into the donor populations, the healthy donor effect, manifesting as a lower mortality among high compared to low frequency donors. This is also comforting considering such donors' extreme risk of iron depletion. Surprisingly, iron depleted donors appear neither to be more prone to infections nor to have a poorer self-reported health.

Within SCANDAT, we have explored the general health effects of blood types. In DBDS, we expand these efforts by extending data collection to include lifestyle factors, circulating biomarkers and genechip data.

Summary/conclusions: Large-scale and extensive data resources accompanied by/also including biobanks are effective tools for scientific investigations answering pertinent questions in transfusion medicine. However, the data structures are also useful for health research beyond transfusion medicine. As such they may constitute a natural extension of the typical blood center function by supplying hospitals and universities with a cost-effective health research infrastructure. To maximize the outcome of transfusion research efforts, extensive international collaboration involving the combination of data sources is highly warranted.

PL3-03

USING BIG DATA FOR EXPLORING OUR UNIVERSE

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Almost all fields of science is experiencing an explosion in the amount of data to be analysed. At the extreme end of this spectrum lies particle physics at CERNs LHC accelerator. In order to maximise the potential to do groundbreaking research, particle physicists is using many forms of machine learning to get results our of what must truly be considered a Big Data analysis. This is the story of how Big Data is used to explore the Universe.

Posters

Management and organisation Organisational issues

P-001

THALASSEMIA CARE IN DHAKA-2016 A RETROSPECTIVE, OBSERVATIONAL STUDY OF CHILDREN ATTENDING IN TWO MAJOR THALASSEMIA CENTERS IN DHAKA, BANGLADESH A LOW INCOME COUNTRIES PERSPECTIVE

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Background: Hemoglobin E β-thalassemia is the commonest form of severe thalassemia in many Asian countries. Overall prevalence of Beta thalassemia trait is 4.1% and Hemoglobin E trait is 6.1% in Bengali school children but little is known about the natural history of the disease, the reasons for its clinical diversity, or its optimal management. Despite its high incidence Hemoglobin E β-thalassemia is often managed in an ill-defined and haphazard way due to its clinical diversity and lack of understanding between genetic variations and disease progression. Management of these patients are therefore usually by demand transfusion. As part of our health link between Barts health and the two major thalassemia centers in Dhaka, Bangladesh we conducted a retrospective study in 50 children attending intermittently for their care

Aims: The aim of this study is to improve collaborations, training and education, exchange of expertise between participating canters. To advocate on safer blood transfusion and transfusion practices

Methods: Retrospective analysis of 50 patients' case records and printed laboratory data were analyzed. Data collected and analyzed include: patient's age, antenatal or postnatal diagnosis, age at diagnosis, type of the thalassemia, diagnostic tests, age at which blood transfusion was commenced, chelation therapy, monitoring, infection, access to specialist/multi-disciplinary clinics, and growth record

Results: The age ranges of the 50 children were between 10 months to 15 years, median age 7 years. 34/50, 68% were HbE β-thalassemia and 32/50, 32% as β-thalassemia majors. The median age of the diagnosis was 4 years. 9/50 children's diagnoses were made by CEA and FBC, remaining 9 were by HPLC. None of the children had mutation/molecular test to confirm the diagnosis. The median age that blood transfusion was commenced was 4 years. 14 children were on hydroxycarbamide of which 3 had β thalassemia Major and the rest are HbE- β thalassemia. 12/50 children were on iron chelation therapy; 3 desferal, 2 GPO-I and rest are on deferiprone. 9/50, 18% children had hepatitis infection of which 7 are Hepatitis C and 2 are Hepatitis B virus infection. All of them have had blood transfusion at different times intermittently. 25/50 children never had a blood test to screen for infections. 5/50 children only had access to specialist review in endocrine and cardiology clinics. Unfortunately 25/50 children's record suggested growth retardation, only 11 recorded as normal and rest of them is unknown. None of these children had antenatal diagnosis; the diagnosis was all made when they attended to these two centers

Summary/Conclusions: There is an immediate need to act on safer blood transfusion to prevent increasing incidence of transfusion related infection. It is clear that the costs of inadequate awareness, screening and management, thalassemia will burden not only the future healthcare budgets of Bangladesh but also the western world in the era of migrating populations. In London, almost all children with Thalassemia are diagnosed by antenatal and neonatal screening, have access to specialist clinics and transfusion related infections are negligible. It identifies the challenges and an opportunity for collaboration between haematologists and policy makers worldwide.

P-002

AUTOMATION IN WHOLE BLOOD PROCESSING: WHAT CAN WE DO?

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Background: As a consequence of technological advances and recent organization methods, it's possible to develop new scenarios in the area of blood component production from whole blood. These new scenarios are necessary to comply with the requirements rose by blood bank centralization and the subsequent increase in blood component production.

Aims: To describe the introduction of different automated devices and lean manufacturing methods in a blood bank processing more than 250.000 whole bold donations in a country with more than 7 million inhabitants.

Methods: We began with the introduction of *Orbisac,* in 2005, to obtain platelet concentrates from buffy coat. In 2007 we implemented Atreus 2C to obtain red blood cells concentrates, buffy coat and fresh frozen plasma. In 2009 we introduced two homemade robots: one to register the characteristics of whole blood units (donation number, weight and temperature) and another to label blood components. In 2011 we changed Atreus 2C for Atreus 3C and in 2013 we implemented Reveos, both addressed to production of red cell concentrates, plasma, platelets and a leukocyte residue.

In 2009 we incorporated an engineer to our staff with the aim of introducing lean methods in the area of blood component production. Lean is a philosophy aimed at increasing the efficiency in the production of blood components and the delivery of services. It is based on the identification and analysis of problems in order to select those activities that add value to the product and the user who, in this case, is the patient.

We evaluated the quality of red cell and platelet concentrates, the number of units lost during production and the working hours needed to obtain blood components from whole blood units between years 2008 and 2016.

Results: The number of whole processed blood units decreased from 287 thousands in 2008 to 253 thousands in 2016. Hemoglobin contain per red blood cell units increased from 52.2 g to 56.0 g. Platelet contain in the average pool unit increased from 2.5×10^{11}, with five units, to 3.1×10^{11}, with four units. The volume of plasma units remained unchanged around 267 ml. The staff's working hours to obtain these products decreased from 25.7 to 17.8 per 100 whole blood units. The percentage of packed red blood cells lost in production decreased from 1.11% in 2008 to 0.81% in 2016. With regard to platelets, the percentage lost in production decreased from 1.2% to 0.3%.

Summary/Conclusions: Automation and implementation of production methods and concepts developed in other industrial areas led to an increase in blood fractionation efficiency and quality.

P-003

Abstract has been withdrawn.

P-004

IRANIAN PROGRAM FOR REDUCTION OF ALLOIMMUNIZATION IN THALASSEMIA PATIENTS

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Background: Transfusion therapy has prolonged the lives of thalassemia patients. One of the major challenges of this conventional therapy is alloimmunization. The presence of alloantibody in thalassemia patients makes the serologic tests difficult, restricts their options to find compatible RBC units and increases the cost. The thalassemia prevention program was approved in Iran in 1995. The results of this program show the downward trend of new thalassemia births. Along with the prevention program of thalassemia, Iran also followed alloimmunization reduction program to manage this phenomenon.

Aims: Due to the critical importance of alloimmunization in transfusion medicine, a systematic review was conducted to evaluate the rate of alloimmunization among thalassemia patients in Iran to follow up the reduction program of alloimmunization in these patients.

Methods: The meta-analysis study was constructed based on the computerized literature database. English and non-English articles were searched from 1994 to 2015. Alloimmunization rates and 95% CI calculated by random effect model. Statistical

analyses were performed using STATA 11.2 and ArcGIS 10.3 was used for map construction.
Results: The result of systematic review was showed the rate of alloimmunization in Iran is 10%. Also we detected the most common alloantibodies (anti-k, D and E respectively) and more prevalent provinces, too.
Summary/Conclusions: Besides the pre-transfusion tests that are done according to blood banking standard of Iranian Blood Transfusion Organization (IBTO), IBTO has started the matching protocol for k antigen of Kell blood group system since 2014 which will continue until 2018. In addition, there is another protocol to Rh system matching specially for D and E antigens. It may lead to have a national program and finally mitigate the alloimmunization rate as low as 70% in thalassemia patients. For achieving the best preventive results in such protocol, it starts in more prevalent provinces.
Performing the screening program to detect the donors with the same phenotype of RBC specific antigens may be helpful to make a pool of donors with the same expression of the most immunogenic RBC antigens such as Kell and Rh other than D antigen. This strategy will be so advantageous while a patient produces an alloantibody against very rare antigen.
Despite RhD matching, antibody against D antigen is high in our country, because through routine serologic examination, variants of D antigens and D-weak cannot be detected. Recently, Blood Group Genotyping Laboratory is established in IBTO, which is the only laboratory in the Middle East that is at the same level of laboratories in developed countries. This specific laboratory facilitates the molecular non-ABO extended matching and help to determine the real RBC genotype. Step by step, Iran is coming to the perfect match and also decreases the rate of alloimmunization significantly.

P-005

IMPACTS OF DEVELOPMENT PARTNERS SUPPORT AND GOVERNMENT COMMITMENT TOWARDS ESTABLISHING SUSTAINABLE BLOOD SUPPLY SYSTEM IN ETHIOPIA

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Background: Ethiopian National Blood Transfusion Service is a humanitarian organization dedicated to save the lives of human being through ensuring availability of adequate & safe blood for all those in need of blood & blood products as part of their treatment since 1969. Ethiopia having dramatically increasing trends of population approaching about 100 million, fast health facility expansion and excellence in quality of care was suffering due to inadequate & inequitable access to safe blood. Since year 2004 CDC PEPFAR Blood safety project were the sole development partner supporting BTS in Ethiopia where challenged by poor grant management system (GMS), no government budgeting & no blood service government ownership.
Aims: To review Impacts of development partners support and government commitment towards establishing sustainable and safe blood supply system in Ethiopia
Methods: A retrospective analysis on level of CDC Blood safety grant awards in the project periods of 2004–2009 and 2010–2016 and review on government grant management system and utilization rate made. The national blood donor database records covering 2005–2015 & review on new national blood service strategy implementation status was also made so as to understand the impact of performance based CDC Blood Safety project budget release and government commitment.
Results: Grant project periods covering 2004–09 and 2010–2016 analysis reveals that the project started with yearly award of 500 USD in the first project period was found challenged by underutilization and poor grant management system with full of restrictions and delayed budget release. The review indicates that the increased government commitment level towards ensuring adequate and safe blood availability initiated by developing new strategy and road map in 2005 and full reversion of the blood service administration from Red Cross society to the government health care system made in 2013. Below are some of the major identified positive impacts of performance based development partner support continuity in Ethiopia:

- Established nationally coordinated and independent government budgeted NBBS institution with initial year budget allocation of 5 million USD in 2014 & reveals the commitment to increase from year to year. The overall CDC grant level increased from 500 USD up to 1500 USD per annum following good performance and proper fund utilization rate of the government.
- Construction of 21 new standard non Hospital based blood bank centres & furnished with standard equipment and trained man power to scale up the 11 centres in 2012 to 25 blood collection, testing, processing and distribution centres. Procurement of 46 new mini blood collection van in 2013 & Increased mobile blood collection teams from 2 to 31.
- Year round awareness creation effort & blood collection increased from 25004 donations in 2004 & 54693 donation in 2012 to 143485 donation in 2016 with increased total voluntary nun remunerated blood donation rate 23% in 2005 and 28% in 2012 to 97.4% in 2015.
- Improved Hospital Blood utilization coverage of safe blood from 45 facilities before 2012 to more than 350 transfusion facilities in 2016.

Summary/Conclusions: Strategic leadership and commitment of the government on National Blood transfusion service through ensuring efficient utilization of grant fund, developing clear strategy towards blood safety and continuity of development partners funding will have a positive impact in establishing sustainable and safe national blood service programme.

P-006

Abstract has been withdrawn.

P-007

LEBANESE RED CROSS BLOOD TRANSFUSION SERVICES: SCALING UP ITS ACTIVITIES TO SATISFY BLOOD NEEDS OF SYRIAN REFUGEES AND HOST POPULATION IN LEBANON

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Background: Blood transfusion in Lebanon relies on hospital- based transfusion centers and on the blood transfusion services of the Lebanese Red Cross (LRC). The LRC is mandated by the ministry of public health to provide health institutions with safe blood. Through its network of 12 branches covering all regions in Lebanon, the LRC is the largest provider and issues some 23'000 units annually free of charge to all citizens. The system is largely relying on family replacement donations. After 2013, the influx of more than 1 million registered Syrian refugees added a big burden on the services. Both quality and availably of blood product was challenged.
Aims: To provide safe blood for the refugees and the host population in Lebanon through improved services.
Methods: Lebanese Red Cross, with technical and financial support of the Swiss Red Cross, started in 2014 a major effort to improve the safety of blood products and to scale up its capacity to meet the needs of the population (citizens and refugees).
The strategy for improvement was twofold: Improvement of quality and safety of blood products and availability of blood products.
Results: By end 2016 and 2 years after the beginning of the project, there is a major progress in terms of quality. All technologists are qualified and had received appropriate training and skill validation. Equipment qualification, and maintenance is done. 7 branches out of 12 are rehabilitated according to WHO recommendations, an FDA approved blood management system is installed and operational, a documentation system is established with a standardization of activities, a quality management system is in place with regular audits, training plan, key performance indicators. The achievements were the basis to reshape the public image of the LRC blood services, i.e. to promote Voluntary Non-Remunerated Blood Donations (VNRBD) and to increase the availability of blood products: In 2016, 1733 Units were collected during regular blood drives and various public events were held. With a new branding identity, a redesign of the website, regular posts on social media and partnerships with institutions; the LRC has laid the groundwork to significantly increase the share of voluntary donations.
Summary/Conclusions: The project has accomplished a major step toward quality and compliance to international standards: the LRC is today providing safe blood to Syrian refugees (25% of all units issued) and host population as well. The next step is to reorganize the blood services so that the LRC can contribute to a larger proportion of the blood components supplied to hospitals and progressively take-up the role of a self-sustaining national blood transfusion service.

P-008

A SITUATION ANALYSIS OF THE BLOOD SERVICES IN PERU

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Background: Pan American Health Organization shows that, in Peru, blood supply is insufficient, with minimal voluntary non-remunerated blood donations (VNRD). In May 2016, the UK Foreign and Commonwealth Office funded a detailed situation analysis.
Aims: To understand the actual situation of the blood services/ blood supply and Transfusion Medicine practice in Peru; share the results of the analysis with the Ministry of Health, Health Services and professionals working in blood banks; inform decision-making to build a national blood programme.
Methods: During two visits (by MC and GP) in August and October 2016, ten hospital blood banks in Lima were visited and discussions held with Peruvian Department of Health officials. A detailed quantitative analysis was undertaken using replies to a questionnaire sent to all hospital blood banks in Peru. The areas covered included: number of donors attending and deferred; number of completed whole blood and component donations; microbiology seropositivity rates; number and type of personnel employed; methods, consumables and equipment used in the collection and testing of donations.
Results: 93 blood banks collect, process and test blood; about one half collecting 1000–5000 units per year, with some collecting < 500 and three >25,000. In Lima, the capital, 37 blood banks collected >65% of the blood supply. Most of the establishments were run by the Ministries of Health or Social Security, but several by the Armed Forces, Police, or privately. Only 6% of blood collected was VNRD, one of the lowest rates in Latin America; the remainder being replacement donations. Over 490,000 prospective donors attended in 2015 throughout Peru, yielding < 313,000 complete donations, i.e. 10 units/ 1000 population. The prevalence of infectious markers in donations was high: e.g. for HIV, 0.3%; for HCV, 0.5%. All blood banks bought different makes of reagents, blood packs and equipment. There was much manual testing, little computerisation, poor quality assurance and no haemovigilance scheme.
Summary/Conclusions: The high number of small establishments, managed by different ministries, meant great variation in standards, procedures and automation levels; it also has prevented opportunity for economies of scale. Blood collection amounts to less than half of what is probably needed in Peru, to ensure sufficiency of supply. The level of infectious markers and poor procedures strongly suggest that the safety of blood transfusion in Peru is sub-standard. Consolidation of blood collection, testing and processing would significantly improve standardisation, efficiency in staffing and reagent costs, allowing investment in the service as a whole. Detailed feedback, with recommendations for change, was given to hospital managers, blood bank directors and the Minister of Health. The response was encouraging. Following the analysis, UK experts in recruitment, donor services, quality, immunohaematology, processing, IT and Transfusion Medicine have visited Lima with the purpose of advising and training staff. A plan for change and consolidation of services is in preparation. This study confirmed the need for expert assistance for developing countries to assess their reality, improve sufficiency, safety, security and efficiency of their blood services.

P-009

Abstract has been withdrawn.

P-010

REPRESENTATION OF DONORS' AND RECIPIENTS' BLOOD TYPES IN MOSCOW

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Background: Planning the production of blood components for megapolis clinics is the main task of the Blood Service (BS). It is necessary to ensure the needs of patients, regardless of seasonal variations in donor activity. On the other hand, the economic situation doesn't allow BC to produce surplus blood components. The occurrence of blood groups in the region is often used for planning the production of red blood cells (RBC), since both donors and recipients are a population of Moscow. According to the population studies in the Moscow region, the frequency of 0 (I) group is 34.3%, A (II) - 36.9%, B (III) - 20.9%, AB (IV) - 7.9%. The basis for planning RBC production is an analysis of the city clinics' needs in general and in real time. However, limiting the activity of voluntary blood donors depending on blood types as well as refusal to patients, is a negative practice. Therefore, it is assumed that in the contingent of donors and recipients the population frequencies of the blood groups should be preserved.
Aims: To determine the possibility of using the population frequencies of blood groups for BS planning.
Methods: The frequency of various blood types in populations of donors and recipients in 2016 was studied, including seasonal variations.
Results: During 2016, the Blood Service of Moscow produced 121025 ready for use RBC units (R-RBCU) from 66627 donors; 49416 patients needed RBC transfusions in 71 municipal clinics, 114084 RBC units were given to them. In general, for 2016, the discrepancy between the representation of AB0 phenotypes in the R-RBCUs and requests for transfusions was 0.8% for 0(I), 0.3% for A(II), 0,6% for B(III) and 0.5% for AB(IV). At the same time, the maximum deviation from the population value of 1.3% was noted for the 0(I) R-RBCUs (Figure 1). Comparing similar data with Rhesus factor (RhD+/-), the discrepancies were more pronounced: from 0.3% to 1.7%, with the maximum discrepancy noted for B(III) RhD(+) (Figure 2).
Taking into account AB0 and RhD phenotypes in 2 monthly periods, we revealed more significant deviations (Figures 3-6). The maximum surplus of ready RBCs was observed in May-June for 0(I) Rh(+) R-RBCUs - 3.7% (1047 units) (Figure 3). The share of ready 0 (I) RBC units was 0.8% higher than the population value, and the share of the requested 0(I) RBC units was 1.7% less (excluding Rh). The surplus units are possibly cryopreserved. The maximum deficit of R-RBCUs was observed in March-April at 0(I) Rh(-) 2% (316 units) (Figure 3). In all cases, the deficiency of RBCs was compensated with cryopreserved RBCs.
Summary/Conclusions: The population frequency of blood types is not advisable to be used as a basis for planning the production of RBCs, since the contingents of donors and recipients are heterogeneous. To prevent the deficiency of certain types, it is necessary to identify the maximum discrepancies in the frequency of the phenotypes of the R-RBCUs produced and requested by clinics in each region. It is advisable to increase production by the corresponding maximum value if there is a cryobank. Otherwise, it is necessary to determine the available model: surplus production with periodic losses of unclaimed R-RBCUs or deficit production with periodic emergency recruitment of reserve donors.

P-011

ORGANIZATIONAL ISSUES IN BLOOD TRANSFUSION SERVICES OF INDIA- A HOLISTIC APPROACH

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Background: Blood Transfusion Service is a vital part of a modern healthcare system. Ensuring access to safe and adequate blood/ blood components to even the remotest part of the Country is the responsibility of the Government. National Blood Transfusion Council is the apex policy making body in India and its key mandate is to ensure accessibility of quality and affordable blood/ blood components through voluntary non-remunerated blood donation to all the people in India. India contributes to nearly 10% of blood collected globally, and two thirds of blood collected in the South East Asia region.
Currently, India has a network of 2,626 functional and licensed Blood Banks widely distributed across 29 States and 07 Union Territories in the Country to fulfil the basic annual requirement of 12.6 million units of blood for civilian population. These Blood Banks are spread across Government, Charitable and Corporate sectors and collect 11.6 million blood units. 73 districts in the country still do not have Government Blood Banks and there exists huge disparity in the availability and access to blood especially in rural areas.
Aims: Blood Transfusion Services of India have a highly fragmented structure and operations with multiple controlling and coordinating Agencies/Departments within the Ministry of Health and Family Welfare such as National Blood Transfusion Council (NBTC), National AIDS Control Organization (NACO), Drug Controller General of India (DCGI) and National Health Mission (NHM) at the National level and similar subsidiary in the States. The lack of coordination amongst these numerous agencies at times acts as a barrier to optimizing efficiency in governance and service provision. There has however been substantial improvement in BTS in India over a period of time, but there are still gaps in ensuring access and availability to affordable, high quality blood and blood products that needs to be addressed at all levels.

Methods: Accurate and updated facility-wise information at the district, state and national level on structural and programmatic components is an essential prerequisite to formulate evidence-based programmes and policies. Therefore, a nationwide assessment in the form of cross-sectional survey was conducted using a self-assessment questionnaire to capture the current situation of all licensed blood banks in the Country. Each component of the questionnaire was given a weight based on the programmatic and quality priorities and scores were allocated to each indicator.
Results: The Assessment results distinctly indicated that an evidence-based, innovative and result-oriented strategies have to be defined to overcome the existing hindrances. The key strategies are targeted towards: developing a nationally coordinated National Blood Transfusion System; ensuring universal access through promotion of voluntary non-remunerated blood donation and rational use of blood/blood components; Institutional strengthening and capacity building; develop and implement quality management systems; promotion of intensive Research and Development in Transfusion Medicine; development of a robust mechanism for referral linkages to care continuum.
Summary/Conclusions: Provision of safe and quality blood for a country like India involves a highly complex operation involving various stakeholders, however the magnitude and complexity of issues raise several challenges. This requires a holistic and comprehensive approach in planning, designing and operationalizing the Blood Transfusion Services in India.

P-012

THE QUANTITATIVE SWOT-ANALYSIS OF ACTIVITY SERVICE BLOOD OF KAZAKHSTAN

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Background: Situation analysis of Blood Service revealed that the strengths dominated over the weak 1.4 times. The possibilities dominate over the threats by 1.1 times. It follows that by using timely the possibility the Service, it is possible to strengthen its strengths and avoid threats.
Priority directions of the resulting analysis is the use of subjective factors, such as personal management capacity managers of the Blood Center in the field of possibilities of legal activity of administrative decisions and the mobilization of stable staff in general market conditions.
Aims: Conduct a situational analysis of the blood service in Kazakhstan and assess its opportunities, risks, as well as strengths and weaknesses
Methods: We used a quantitative SWOT analysis.
Results: We have studied for the period 2011–2015 retrospectively indicators of Blood Service of Kazakhstan.
Strengths of Blood Service of RK(Ps4,5): 1) indicators of donations for 1000 people is higher than in some countries of the CIS (in Russia 14, Kazakhstan - 17); 2) the functioning science centre of transfusiology; 3) the legal possibility of additional financing blood centers in the form of paid services; 4) the absence of competitors; 5) training on specialty Transfusiology; 6) the stable management staff of blood centers, a high level of competence; 7) increase in the incidence requiring transfusion therapy (oncohematology); 8) equipment upgrades; 9) the opening of a reference laboratory; 10) growth of the proportion of issued high-quality blood components; 11) governmental organization "Drop of Life"; 12) the functioning bone marrow register; 13) equipped with modern equipment HLA-laboratory; 14) High categorization of average medical workers; 15) availability of own specialized scientific journal; 16) vivarium.
The weaknesses (Ps = 3.5): 1) the incomplete equipping of blood centers and blood transfusion cabinets according to standards; 2) low specific weight of paid services of the CC; 3) low categorization of doctors; 4) high staff turnover; 5) the low scientific potential of industrial and clinical of transfusiology; 6) the low wages paid; 7) slight growth of harvested donor blood; 8) the weak continuity with offices blood transfusion; 9) lack of agitation work; 10) the formally functioning of Transfusion Association; 11) the absence of scientific laboratories; 12) high standard deviation from the average for donations in the republic.
The possibilities (Ps = 2,7) 1) international grants; 2) organization of international seminars on clinical of transfusiology; 3) cross-sectoral cooperation; 4) to increase scientific research in the field of clinical of transfusiology; 5) reduce the share of donation unsuitable for transfusion and processing; 6) strengthening marketing management in the development of fee-based services; 7) advertising paid services; 8) strengthening the publishing of teaching aids; 9) team-building staff.
The threats (Ps = 2.1): 1) an increase of staff turnover; 2) the risk of litigation; 3) reduction gemoprodukts based on evidence-based medicine; 4) regression of scientific capacity; 5) organization of marketing; 6) transfusiologist marketing; 7) reduction in the quality of the evidence base transfusions.
Summary/Conclusions: Strengths prevail in the weak and opportunities over the risks of 1.3 times.

Information technology

P-013

IMPLEMENTATION OF COMPUTERIZED BLOOD BANK MANAGEMENT SYSTEMS IN FIVE DEVELOPING SETTINGS: LESSONS LEARNED

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Background: There is growing implementation of computerized blood bank management systems (CBMS) in developing countries. The Swiss Red Cross (SRC) had partnered a number of countries mainly in Africa in the implementation of their CBMS. To date the successful completion of CBMS projects has been achieved in Zimbabwe and Eritrea and are ongoing in Egypt, Malawi, and Lebanon. Based on the project completion and progress status reports there are a number of lessons learned that will help similar projects to take note of as presented in this review.
Aims: The aim of this study was to document and share the lessons learned in implementing Red Cross funded CBMS projects in five developing countries.
Methods: The countries that had implemented a CBMS under the support of SRC were identified and their project status noted. There were five country projects, and the study considered CBMS projects that had either been commissioned (Zimbabwe and Eritrea) or are at an advanced stage of implementation (Egypt, Malawi, and Lebanon). Progress status or final reports for these CBMS were retrieved and lessoned learned abstracted. The lessons learned were assessed from the three different stakeholder perspective of the vendors (suppliers of the CBMS), funders (SRC) and the customers (blood services/centers). Key issues considered were the tendering process, project cycle management, user specification requirements, data migration, budgets, and hardware and communication requirements.
Results: The tendering processes including specifications and selection of a supplier was successful in all countries and this led to the right product for the respective settings. Project Cycle Management went well, where clear roles and responsibilities were defined and not too many actors were involved. During implementation, the biggest challenge for all implementers was to the user requirements specification and the subsequent adaption process. In Egypt, Malawi, and Zimbabwe this step has taken much longer than planned. Data migration was a challenge for countries such as Zimbabwe that were previously working with a homegrown legacy system (since 1994) and to change over to a new system (commissioned in 2012) that was following certain quality assurance standards. In Malawi and Zimbabwe, the feasibility studies before launching the project were not comprehensive enough, especially in regards to the hardware- and communication infrastructure investments, which were underestimated. Although all three ongoing project face technical difficulties, the projects are progressing though delayed and the investments are higher than originally planned. The two countries with completed projects report a high satisfaction with their operational CBMS.
Summary/Conclusions: CBMS implementation was a necessity not only for work efficiency but also for improving blood safety for all the developing country settings. The study findings indicate the need for more exchange and availing of practical CBMS tools and aids to blood services in low-and middle-income countries. The AfSBT has initiated the setting-up of the IT working group, which needs to be supported to address these expectations.

P-014

MINIMISING HUMAN ERRORS IN BLOOD BANKING USING INFORMATION AND COMMUNICATION TECHNOLOGY

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Background: Blood banking requires repetitive actions at various steps involving multiple personnel, making the process error prone. In addition, high volumes as-

well as variable human resource have a compounding effect. Furthermore, the consequences of these errors can be serious. Hence, strategies of pro-active prevention of human errors need to be identified.
Aims: To investigate the effectivity of Information and Communication Technology (ICT) in intercepting common human errors in blood banking.
Methods: The Blood Bank Management Information System (BBMIS) developed in the Indus Hospital Blood Centre (Karachi, Pakistan), has been designed to intercept critical human errors in real time, by embedding truth tables and decision trees for each process control point. It informs the user by generating an on-screen alert while blocking further user activity till the error is corrected. In addition, it notifies the admin of the "error" prevented. Error alert frequencies for the period of 37 months (Dec 2013- Dec 2016) were extracted from the BBMIS database.
Results: Out of 136,545 blood components prepared, 116 units initially had a wrong expiry label pasted on the bag. Hence on average for every 1177 components prepared, there was 1 wrong expiry label pasted. All 116 errors were prevented.
Donor blood group testing was performed on 45,515 blood donors for which 57 alerts of "donor blood group mismatch" were generated at donor group reconfirmation. Hence on average for every 798 blood group reconfirmations, there was 1 erroneous blood group judgement. All 57 errors were prevented.
A total of 50,364 patient samples were processed and 126 alerts of "patient blood group mismatch" were generated indicating incorrect patient sample either at draw or by the blood bank technologist during testing. Hence, on average for every 397 patient samples, there was 1 incorrect patient sample error. All 126 errors were prevented.
A total of 123,978 blood groups were performed, out of which 169 alerts of "misinterpreted blood groups" were initially generated. Hence on average, for every 733 blood groups performed, 1 was misinterpreted. All 169 misinterpretations were prevented.
A total of 141,452 units underwent e-unit verification, for which 98 alerts of "incorrect blood group label pasted" were generated by the BBMIS. Hence, on average for every 1443 blood group labels pasted, 1 was incorrect. All 98 errors were prevented.
Summary/Conclusions: ICT is an effective tool for the identification and prevention of "Human Errors" in blood banking. Human errors can be identified by comparing their actions at each step of the process against truth tables and decision trees specific for that step. Deviations from the truth tables and decision trees are indicative of a violation of a rule. Hence, when used intelligently, ICT enables processes to be followed consistently in real time, thus ensuring delivery of safe blood.

P-015

THE IMPACT OF COMPUTERIZED PROVIDER ORDER ENTRY (CPOE) WITH CLINICAL DECISION SUPPORT (CDS) UPON UTILIZATION OF BLOOD COMPONENTS: USAGE REDUCTION OR ALERT FATIGUE?

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Background: Computerized Provider Order Entry (CPOE) offers the potential for safer and faster patient care, and better compliance of evidence therapy. However, the effectiveness of CPOE with clinical decision support (CDS) on blood components usage remains controversial.
Aims: To evaluate the effect of CPOE with CDS upon blood components usage.
Methods: The CPOE with CDS for plasma and platelet (PLT) usage had been applied since 2010 in a tertiary hospital with 1200 beds. An alert reminding mechanism based on the threshold for respective blood components was defined and issued by the local transfusion committee was integrated to the system simultaneously. Subsequently, red blood cell (RBC) usage had also been incorporated since 2015. The amount of respective blood components during the period before (pre-CPOE) and after incorporation of CPOE (post-CPOE) were analyzed and compared.
Results: During the study interval from 2008 to 2016, a total of 138,086 plasma products and 507,477 platelet products were analyzed in 2,542,338 patient-days; a sum of 151,995 RBC products in 1,138,542 patient-days from 2013 to 2016 was also analyzed. The average units for plasma usage transfusion was 51.1 $\pm$ 4.2 and 56.0 $\pm$ 4.3 units per thousand patient-days (P = 0.173) in pre- and post-CPOE periods, respectively. Correspondingly, the average PLT usage for pre- and post-CPOE periods was 182.5 $\pm$ 18.4 and 208.9 $\pm$ 21.8 units per thousand patient-days (P = 0.117), respectively. Furthermore, the average units of RBC transfusion for pre- and post-CPOE periods was 137.7 and 126.2 units per thousand patient-days (P = 0.053), respectively.
Summary/Conclusions: Introduction of COPE with CDS seemingly decreased RBC usage, though there was no statistical significance. The effect of COPE upon PLT and FFP usage seemed to be negligible. Our results implied that CPOE may partially help reducing blood components usage in the very beginning, but alert fatigue could appear in later over an extended period of time.

P-016

THE RIGHT BLOOD FOR THE RIGHT PATIENT: BRINGING INNOVATION AND TECHNOLOGY TOGETHER

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Background: The risk of receiving a wrong blood unit depends on the level of control over critical steps that include obtaining samples, patient identification, unit allocation, and blood product administration. Laboratory Information Systems (LIS) and Electronic Medical Record (EMR) separately control some steps of the transfusion process, but generally do not offer comprehensive control over the entire transfusion process.
Aims: Our transfusion service was launched with separate IT systems for the EMR, LIS and remote blood inventory management. Under these circumstances, we sought to improve patient safety by bridging these systems to provide comprehensive transfusion process controls
Methods: Controls were needed for transfusion appropriateness, patient identification and specimen collection, removing of blood products from peripheral refrigerators, and bedside blood administration. An interface was available between the EMR (Epic Systems Corporation, Verona, WI), Collection Manager System (Epic Systems Corporation, Verona, WI) and the LIS (Sunquest Information System, Tucson, AZ). A unidirectional print-capture interface was available between the LIS and Haemonetics BloodTrack Courier for control of peripheral refrigerators.
Results: We have made different innovations in medical informatics and process of transfusion chain by designing integration of four different IT systems. Justification of Blood Transfusion:. A Best Practice Alert (BPA) was created in Epic and is triggered when an order is placed and there is no Complete Blood Count (CBC) or coagulation result (INR in case of plasma) in the last 24 h, or when the hemoglobin is above established transfusion guidelines/ Patient Identification and Specimen Collection*:* Flow of information from EMR to CMS and finally to LIS in real time, allow anyone to look into the status of specimen collection in all three IT systems. Blood Bank Compatibility Test: Once the compatible units have been allocated for a defined patient, this information comes across from Sunquest (SQ) LIS to the Epic EMR. Remote Issuing of Blood from Peripheral Fridges*:* The information flows from LIS to BloodTrack in order to maintain traceability. Administration of Blood: At this point all interactions of the IT systems come together to ensure the right blood unit is being given to the right patient. Any mismatch in the scanning of the next alerts caregiver with a hard stop: ABO Type of Unit; Donor Identification Number; Product Code and Expiration Date
Summary/Conclusions: Safety in transfusion depends on the expertise of all different stakeholder involved in the process. While the IT systems help in many of the steps of the process, in our opinion, the integration of different IT systems is essential to ensure that the information is readily accessible to all caregivers involved in the transfusion continuum. The system design should allow for early and easy detection of low frequency errors that may have an adverse impact on the patient and would otherwise be difficult to detect with non-interacting workflows. We expect these innovations to be a useful new tool in global healthcare quality and patient safety.

P-017

PRECODED BLOOD SAMPLE TUBES IMPROVES BLOOD SAFETY

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Background: Usual blood donor samples were labeled at the donation side before venipuncture. In case of unsuccessful venipuncture re-labeling of samples tubes might be necessary. In the blood donor service Baden-Wuerttemberg- Hesse two

donors were donating blood side by side separated by a table in the middle of two beds. Mix up failures between both donation occurred approximately 2-3 times per year and will be identified in the laboratory by blood grouping.
Aims: Pre-coded sample tubes from Greiner Bio One were used in a pilot study to exclude all mix up failures at the blood donation side. Therefore an alpha-numeric code was labeled by the sample tube manufacturer on the tubes. All tubes were scanned at the blood donation side before filling and merged together with the blood donation number on the sample bags. In the laboratory all numbers (donor number, donation number and precoded sample tube numbers) were handled by the laboratory information system. After testing all test results were transferred into the blood donation program (Inlog Edge blood).
Methods: All samples were screened by serology methods with ABBOTT PRISM for HBsAG, Anti-HCV, HIV combo and Anti-HBc and in parallel by NAT with the Roche Cobas MPX and DPX assay.
Results: In total three first time donors with three different precoded samples tubes per donation and six multiple time donors with two different precoded samples tubes were tested in the pilot study. All sample tubes were scanned and electronic merged together with the blood donation numbers. Testing at all instruments (preanalytic decapper Sarstedt, Beckman Coulter, Hamilton pooling instruments, Roche Cobas 6800 and 8800, ABBOTT PRISM, and Beckman Coulter PK7300 instruments) was able without any specifications. After testing data transfer from the LIS into the blood donation programme was in addition possible without any failures.
Summary/Conclusions: The pilot study demonstrates that the introduction of pre-coded sample tubes with alphanumeric bar-codes is feasible and able to improve blood safety in order to prevent any sample mix ups at the blood donation side. The use of pre-coded sample tubes reduce the electronic devices at the blood donation sides and optimize scanning of sample tubes in the laboratory because all barcodes were labeled exactly at the same place. Finally all samples tubes were linked to the blood donation bag immediately before filling the samples tubes. Using of pre-coded samples tubes reduces the risk of manual failures and improves laboratory testing on automated screening instruments. Therefore the blood donor service Baden-Wuerttemberg – Hesse and North-East will implement pre-codes sample tubes for all donations in 2017.

P-018

VALIDATION OF MULTISOURCE ELECTRONIC HEALTH RECORD DATA: AN APPLICATION TO BLOOD TRANSFUSION DATA

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Background: Although data from electronic health records (EHR) are often used for research purposes, systematic validation of these data prior to their use is not standard practice. Existing validation frameworks discuss validity concepts without translating these into practical implementation steps or addressing the potential influence of linking multiple sources.
Aims: To develop a practical approach for validating routinely collected data from multiple sources and to apply it to a blood transfusion data warehouse to evaluate its usability in practice.
Methods: The approach consists of identifying existing validation frameworks for EHR data or linked data, selecting validity concepts from these frameworks and establishing quantifiable validity outcomes for each concept. The approach distinguishes external validation concepts (e.g. concordance with external reports, previous literature and expert feedback) and internal consistency concepts which use expected associations within the dataset itself (e.g. completeness, uniformity and plausibility). In an example case, the selected concepts were applied to a transfusion dataset and specified in more detail.
Results: Application of the approach to a transfusion dataset resulted in a structured overview of data validity aspects. This allowed improvement of these aspects through further processing of the data and in some cases adjustment of the data extraction. For example, the proportion of transfused products that could not be linked to the corresponding issued products initially was 2.2% but could be improved by adjusting data extraction criteria to 0.17%.
Summary/Conclusions: This stepwise approach for validating linked multisource data provides a basis for evaluating data quality and enhancing interpretation. When the process of data validation is adopted more broadly, this contributes to increased transparency and greater reliability of research based on routinely collected electronic health records.

P-019

MOVING WITH THE TIMES: ADOPTING DIGITIZATION TO IMPROVE WHOLE BLOOD COLLECTION PROCESSES IN SINGAPORE (PHASE 1)

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Background: The daily operations of Singapore's blood bank have always been heavily reliant on manpower and manual documentation to cross-check all stages of collection. However, with a growing demand for Whole Blood every year, it is no longer feasible to do manual monitoring of each step of the collection process. Moreover, with increased documentation and collation of data, it is also not time- or work- efficient. Hence, adopting digitization and customizing software to meet the blood bank's collection protocols will yield manifold advantages such as:
- Reducing manual documentation and transcription errors.
- Ensuring accuracy and enhancing process controls.
- Providing electronic data management.
- Augmenting blood product safety with donor ID, user ID and ISBT labeling checks.

Aims: To ensure standardized collection processes and good practices, safeguarding both donor safety and safety of blood and ensuring efficient use of manpower.
Methods: Using Terumo BCT's T-RAC II blood mixer-devices together with Terumo Operational Medical Equipment Software (TOMEs) created a total blood collection process system with electronic data management. The Singapore blood bank embarked on a two-phase journey to build an automated, quality blood collection infrastructure, integrating blood collection mixer-devices with electronic data management system. Phase 1 comprised of a pilot project interfacing 32 units of automated T-RAC II blood mixer-devices in the blood bank's headquarters with TOMEs. While Phase 2, planned for the near future, will deploy the T-RAC II mixer-devices across all other satellite collection sites with integration to TOMEs and the Blood Bank Management System - eProgesa.
In Phase 1, the mixer-devices "communicated" with TOMEs electronic data management system through local area network (LAN) linkage. This allowed for further configuration of mixer-device functions to the blood bank's process requirements. Additional functions such as input of bag reference and lot numbers, operator and phlebotomist barcode numbers and incidents encountered during the donation process were also included.
Results: Mixer-device connectivity to software and user computers for viewing or printing of session data faced an initial drawback due to firewalls encountered in the blood bank's system and the need to adhere to the government's Internet separation directive. From mid-2017, all public services' computers will be delinked from the Internet, a move by the Singapore government to ensure cyber security and safeguard against attacks on individual public service officer's computer. This directive would rule out the use of WIFI or Web browsers but was resolved through LAN linkage. Staff feedback from the pilot cited user-friendliness, confidence in quality monitoring and uniform performance.
Summary/Conclusions: Phase 1 linkage of blood mixer-devices to a dedicated electronic data management system is expected to reduce the risks associated with errors through manual documentation and monitoring. Despite initial effort needed, the blood bank recognized the need to go beyond just automation but also for consistent real-time monitoring and replace both manual and paper processes which only digitization can afford. With the success of this pilot, the blood bank will scale upwards to Phase 2 link-up, which will ensure seamless communication between the blood bank management system and all interfaced mixer-devices across all sites.

P-020

VALIDATING THE MIGRATION OF AN AUTOMATED BLOOD MANAGEMENT SYSTEM

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Background: The Egyptian National Blood Transfusion Services (ENBTS) has started the soft opening for the Automated Blood Management System (BMS) at National Blood Transfusion Center (NBTC), its headquarter, by mid August 2016. During the first 6 month of implementation we reported 81 errors in software (68), hardware (2), medical equipment communication (6), barcode printing (1) and mobile tool (4). By that date we had 28,000 donor registered to the BMS.
Based on the above errors a new version of the software and database (DB) were introduced by the software provider with solutions to the software errors.

Aims: This is a quantitative research that describes the steps we used to validate the data migrated from the old database (DB1) and matching it with the data on database (DB2), thus ensuring that data is intact and was not lost. Based on the success of this validation, NBTC will take the decision of migrating or having to delete the whole database and starting from scratch.
This is a quantitative research that describes the steps we used to validate the new database (DB2),matching it with the old database schema (DB1),thus ensuring that data is intact,and was not lost during the final migration between the two databases.
Methods: The Software Provider was responsible for migrating the data from the DB1 to DB2. The database used was Microsoft SQL Server (MSSQL) 2012. While NBTC Information Technology Department (ITD) members were responsible for validating the transferred data.
The ITD took backup from DB1 and DB2 using MSSQL2012, before proceeding with the validation. ITD team used the 'Microsoft Visual Studio (MSVS) Data Compare tool' to validate the migration process.
This was achieved through selection of DB1 as a Source Database and DB2 as a Target Database. The software then was capable of running a simultaneous comparison between the two databases.
Results: The 'Data Compare Tool' showed that all the 739 tables in DB1 were transferred correctly to DB2 except for four tables, the most significant of them were:

- Data available only in the source DB1: 66 records related to donation separation were missing on DB2 out of 82000 records. Also, there were 3 records from the unit lock table were missing.
- Data changed when transferred to DB2: There was only one record out of 28000 in the donors table where the Arabic letters changed to '?????'. Also, there was 1 record in the system tables that was updated with the software version.
- Data that was deleted in the destination DB2: There were 24 records related to user tracking that went missing after the migration.

Summary/Conclusions: It was very vital for us to keep the data collected in the past 6 month. The validation of this data eliminated any risks attached to deleting the entire database and having to start again from scratch thus saving time and effort.
Our study showed that more than 90% of the data was transferred successfully. However some of these data loss/changes are acceptable and others are not. For example, the 66 missing separated units, this is a critical issue that was immediately reported to the Software Supplier to solve, since it has an impact on the donor status and ultimately on the safety of the blood.

P-021

IMPLEMENTATION OF AN ELECTRONIC ORDERING SYSTEM FOR BLOOD PRODUCTS

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Background: In order to optimize the ordering of blood products, a computerized physician order entry (CPOE) system was implemented at a tertiary care hospital in December 2016. The service is available as part of the electronic patient record. The CPOE displays the patient's hemoglobin concentration, platelet count and INR within the 48 h prior to the time the order is placed. The ordering prescriber must fill in a variety of information including the requested blood product(s) and their indication (s). If the indication for transfusion is not in accord with the established national guidelines, an alert appears. The prescriber can then either cancel or force the request. In order to initiate plasma thawing, a plasma order has to be followed by a call to the blood bank.
Aims: To document the implementation of electronic ordering related to time of day and type of product. To determine within the electronic orders the use of the indication Anemia in relation to the hemoglobin concentration.
Methods: Data on all blood product orders from 1 week, 5 weeks after implementing the system were retrieved from the blood bank database (ProSang, Databyrån AB, Stockholm, Sweden). Statistics: Chi square test.
Results: A total of 565 blood components were ordered. Out of these, 388 (69%) were placed through the CPOE and 177 (31%) were placed manually.
The CPOE was used to place 74% of the blood product orders during the day (0701-1500), 68% of orders during the evening (1501-2300), and 49% of orders during the night (2301-0700); ($P < 0.001$, night vs. others).
The CPOE was used to place 77% of the orders for red blood cells (RBC), 75% of the platelet orders and 38% of the plasma orders; ($P < 0.001$ plasma vs. others).
When ordering RBCs through the CPOE, the indication *Anemia* (hemoglobin concentration <4.3 mmol/l (7 g/dl) was used in 127/272 requests (47%). In 18/127 (14%) of these orders the national guideline hemoglobin threshold was met; in 78/127 (62%) of the RBC orders the hemoglobin concentration was above the threshold, and in 31/127 (24%) orders the patient's hemoglobin concentration had not been measured in the preceding 48 h.
Summary/Conclusions: Overall the rate of ordering blood products using the CPOE is 69%. The absence of total compliance with its use might be in part due to trauma situations, and likely the prescribers' overall unfamiliarity with its use. Electronic ordering is used more frequently during the day, than in the evening and night. This could be due to insufficient training of physicians/nurses working these shifts or due to differing work routines according to the time of day. The mandatory order confirmation by phone for plasma likely explains why only 38% of plasma component orders are placed using the CPOE. That only 14% of RBCs ordered for Anemia were actually on patients whose hemoglobin concentration was <4.3 mmol/l (7 g/dl) indicates that this indication is being overused and that education efforts must be focused on the prescribers who are using this indication erroneously.

Cost/effectiveness

P-022

CHALLENGES ON FINANCING BLOOD TRANSFUSION SERVICES IN DEVELOPING COUNTRIES: EXPERIENCE FROM SENEGAL (2006–2016)

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Background: Development and implementation of a budgeting and finance system is a prerequisite to ensure a sustainable and well established blood transfusion service. Making all the funding sources under control is essential to reach this goal. In the strategic plan for BTS in Senegal, one point was to implement a sustainable financing model for blood transfusion service. In this purpose, 4 activities were planned: to work out a uniform pricing of the blood products for cost recovery, to develop the partnership with NGO, financial and technical partners, to advocate the authorities on the need for allocating more resources and to develop income generating activities
Aims: The aim of this study was to evaluate the evolution of funding sources of blood transfusion services from 2006 to 2016 and to discuss challenges and success
Methods: We analyze the source of funding of BTS budget from 2006 to 2016 and compare the contribution from the different sources: Government contribution, international partners, cost recovery, own services activities, private public partnership and miscellaneous. We also analyze the observed challenges and identify new perspectives to a better sustainable model of financing.
Results: During this period, the main 3 sources of funding were: government contribution, own services activities and international partners with equal proportions of 30 to 35% each. This funding model was stable and few variations were seen in these 3 funding sources during these last ten years. In 2016, reduction of international partner's contribution has resulted to a risk of disturbances and actions had been made to tackle this new challenge. Public private partnership was used temporally in 2005 and 2014 to resolve an urgent investment need for costly equipments. The main encountered challenges were difficulties to convince the government to have more resources, the high claims to be recovered from our customers, administrative delays and NBTS participation to budget heading with the international partners, constraints with tendering laws when considering private public partnership and low contribution from local collectivities and philanthropists to support blood transfusion activities.
Summary/Conclusions: The different sources of funding for blood transfusion activities were stable during this last decade and the identification of different challenges will help to address new perspectives.

P-023

SIMPLE INITIATIVES TO REDUCE BLOOD WASTAGE IN A PAEDIATRIC TERTIARY HOSPITAL

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Background: Blood product waste is an important issue in major hospitals. An initiative was taken as part of the patient blood management program of my hospital. The causes and the amounts of wastage were identified. Simple, cost effective targeted interventions were implemented to reduce the wastage.
Aims: The aim of the study was to monitor the wastage and note if simple interventions could reduce the wastage of blood products in subsequent periods of time.
Methods: The blood wastage in pediatric operation theatres of my hospital was monitored. The blood bank was instructed to supply the details of every unit of blood product wastage to a single consultant anesthesiologist. The audit was conducted by this consultant anesthesiologist using lean sigma process. This included defining, measuring, analysing, controlling and improving the outcomes. The consultant anesthesiologist conducted the audit for every wastage of blood product which included finding the cause, communication with concerned staff members and suggesting ideas to prevent such wastage in future.
Results: The audit was started in Jan 2013. The rate of wastage of blood product was 0.9%(6/665) in 2013; 0.78%(5/635) in 2014 and 0.81% (6/735) in 2015. In 2016, there was zero wastage of blood products (0/720).
There was no cost involved in this audit.
Summary/Conclusions: Simple interventions like audit using lean sigma process can have a dramatic impact on reducing blood wastage with regard to both cost and resource saving.

P-024

ANALYSIS OF REQUESTING, DELIVERING AND RETURNING OF RED BLOOD CELL CONCENTRATES IN BLOOD TRANSFUSION CENTRE, FACULTY OF MEDICINE, KHON KAEN UNIVERSITY, THAILAND

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Background: Red blood cell concentrates (RCs) must have compatibility testing. Its lead to work load and quality of lab management for deliver and return RCs. Also, the waste of unit cost will be discussed.
Aims: To analysis rate of requesting, delivering, and returning of RCs. at Blood Transfusion Centre, Faculty of Medicine, Khon Kaen University, Thailand.
Methods: The data of patients who request RCs, were collected and manual recorded into Excel.
Results: N of analysis is 961 patients, divide into blood group A, B, O and AB is 194, 320, 337 and 90 cases, respectively. The RCs requested were 389, 444, 460 and 144 units per blood group, respectively. Total number requested were 1,645 units, delivered to wards were 1,371 units and returned to blood bank were 617 units.
Summary/Conclusions: The data was shown requesting too much .The excess request is over 1,028 (request 1,645/return 617) units. The compatibility test done 1,371 units and delivered, but the return was 617 units. Therefore the C/T ratio was 1.8. This analysis present to waste or excess of RCs supplies about 80%. Then we should discuss and plan for decrease work load and excess RCs with the medical team.

P-025

LONG-TERM MONITORING OF WASTED TRANSFUSION PRODUCT

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Background: Historically, hospital administrators have viewed the transfusion service as an escalating, out-of-control cost center. Birth of component therapy and the invention of plastics in the 1990s improves patient care in the Czech republic too. Simultaneously with this improvement the costs greatly increased. Transfusion and Tissue Department of Brno Faculty Hospital is institution with production more than 35 000 donations per year. Quantity of wasted blood products is one of monitored parameters to verify a functional inventory management and to provide the service more effectively.
Aims: The aim of our work was analysis of the quantity of the wasted transfusion products according to the types (red cells, platelets, plasma) during period 2001–2016. Quantity of wasted transfusion product was expressed as percentage of annual total production of particular type.
Methods: All blood products taken out of the store are classified as discarded (by reason of reactivity, damage or failure of recommended quality) or outdated.
Results: The quantity of wasted transfusion products for all monitored types got around 3% during the first part of period and it declined over the period to 2% for red cells and platelets respectively 1% for plasma due to corrective actions by the Transfusion and tissue Department.
Summary/Conclusions: Analysis of wasted blood products showed us some spares in the inventory management in Transfusion and Tissue Department. Minimization of the wasted blood products is one of the steps how to provide the blood inventory management as effectively as it is possible.

Training and education

P-026

TEACHING WITHOUT BORDERS: USING ELEARNING TO IMPROVE CLINICAL TRANSFUSION PRACTICE IN THE DEVELOPING WORLD

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Background: BloodSafe eLearning Australia (BEA) (www.bloodsafelearning.org.au) is an online educational program designed to increase knowledge of patient blood management and clinical transfusion practice. The program has approximately 400,000 registered learners who have completed more than 760,000 courses.
While the program is aimed at, and promoted to, Australian healthcare workers all courses are freely available on the internet, anywhere in the world. To date no analysis of the uptake or applicability of the program to an international audience (outside of Australia) has been undertaken.
Aims: To analyse the uptake, usage and feedback on BEA courses by international learners with a view to determine if there are identifiable changes required for an international audience, particularly in the developing world.
Methods: A retrospective analysis of learner registration data, course completion records and course evaluation questionnaires was undertaken to investigate the uptake, usage and feedback by international users.
Results: Analysis of registered international learners shows that as of 31 December 2016 there were 7,560 registered learners from countries outside of Australia, who had completed 10,864 courses.
These learners came from 178 countries. Breakdown of learners' countries by the Human Development Index (HDI) shows that:

- 4743 (62.7%) learners live in very high HDI countries eg New Zealand, USA, UK, Singapore, Canada.
- 1040 (13.8%) learners live in high HDI countries eg Malaysia, China, Sri Lanka.
- 1372 (18.1%) learners live in medium HDI countries eg India, Philippines, South Africa, Cambodia.
- 316 (4.2%) learners live in low HDI countries eg Nepal, Pakistan, Afghanistan.

Learners include nurses or midwives (57.9%), medical officers (24.2%), laboratory staff (9.7%) with the remainder (8.3%) coming from other clinical and non-clinical areas including administration, paramedics and the university sector.
Quantitative and qualitative analysis of learner evaluation questionnaires showed that 93.5% of respondents believe the course/s provided them with increased knowledge and 63.6% can make changes to their clinical practice based on this knowledge. Free text comments showed the majority of users believe the courses are of high quality, relevant and up-to-date. One challenge identified by a small number of users included the need for English language literacy, with a suggestion to make greater use of a glossary to assist with word meanings.
Summary/Conclusions: This analysis of a small number of users demonstrates that elearning can be used to provide consistent, credible and reliable education on transfusion medicine knowledge, practice and governance to healthcare professionals anywhere in the world.
While there will be challenges to overcome including language and literacy, terminology, and customisation to locally available blood products and laboratory testing, the use of elearning provides an opportunity to improve transfusion medicine on a large scale in a very cost-effective manner.

P-027

THE ROLE OF ELEARNING IN IMPROVING TRANSFUSION PRACTICE AND IMPLEMENTING PBM

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Background: BloodSafe eLearning Australia (BEA) (www.bloodsafelearning.org.au) is an online educational program to increase knowledge of patient blood management and clinical transfusion practice for Australian healthcare workers. These courses provide consistent, key messages with learning aligned to Australian Patient Blood Management (PBM) Guidelines, National Safety and Quality Health Service (NSQHS) Standards, Australian and New Zealand Society of Blood Transfusion guidelines, published evidence and expert consensus. They are designed to inform improvements in clinical practice and improve patient outcomes.

BEA has developed and delivers 15 courses that are self-paced, follow adult learning principles and support user-selected learning pathways. Courses utilise a range of learning materials including videos of experts discussing and/or demonstrating best practice, interactive learning objects and case scenarios to provide a flexible, authentic learning experience. Learners can apply and test their knowledge by completing activities and clinical-based scenarios. All courses include a formal assessment, and a certificate is provided upon successful completion. Many Australian hospitals, universities and other organisations mandate/recommend that their staff complete one or more courses in order to enhance PBM and transfusion safety and to assist their organisation to meet the NSQHS Accreditation Standard 7 for Blood and Blood Products.

BEA is funded by all Australian Governments via the National Blood Authority. All courses are available at no cost to the learner or their hospital/organisation.

Aims: To undertake a formal evaluation of the courses in order to determine if the program is meeting its objectives of providing knowledge that can be put into practice in order to improve patient outcomes.

Methods: A retrospective, qualitative and quantitative analysis of course completion questionnaires was undertaken to investigate the practical use, perceived quality, value and effectiveness of the BEA courses and how this affects learners within the Australian clinical context.

Results: Analysis of learner registration and course completion data shows that as of 31 December 2016 the BloodSafe eLearning program has 399,468 registered learners, who have completed 769,997 courses. Approximately 14,000 courses being completed each month.

Analysis of 3,885 learner evaluation questionnaires showed that users believe the program: increases knowledge (89.3% of respondents), changes clinical practice (61.73%), assists to identify near misses and adverse events (83.6%) and improves patient outcomes and safety (87.6%).

Actions and changes identified by users that improve outcomes and safety include: reviewing local policies to align with guidelines, more appropriate use of blood, development of massive transfusion protocols, better assessment and management of postpartum haemorrhage, and providing staff with education and mentoring in transfusion practice.

Summary/Conclusions: Analysis of user evaluation data demonstrates that BEA has played an important role in improvements to clinical transfusion practice and patient blood management in Australia. The program provides users with credible, consistent knowledge that they can apply to their clinical practice. This model of online education can be applied more broadly to other contexts, and could be used to improve transfusion knowledge, practice and governance in the developing world.

P-028

SIMULATION TRAINING IMPROVES KNOWLEDGE AND CONFIDENCE OF JUNIOR DOCTORS IN THE MANAGEMENT OF TRANSFUSION REACTIONS

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Background: Pulmonary complications of transfusion (TRALI-Transfusion Related Acute Lung Injury, TACO – Transfusion Associated Circulatory Overload, TAD – Transfusion Associated Dyspnoea) are dangerous and result in the greatest number of transfusion-related deaths as reported by the UK Haemovigilance Scheme Serious Hazards of Transfusion (www.shotuk.org). Although blood transfusions have the potential to cause severe morbidity and mortality, previous studies have identified junior doctor education in transfusion practice as unsatisfactory and recommended an emphasis on face to face training and simulation. Simulation training provides a safe and controlled environment to teach a wide variety of skills and in Transfusion Medicine has been used in particular in the field of major haemorrhage and resuscitation. Accordingly, we have developed a simulation program for the recognition and management of acute transfusion reactions and undertaken an assessment of its outcomes on junior doctor training.

Aims: To assess the effectiveness of simulation training in the management of transfusion reactions for junior doctors, using a pre-test, post-test evaluation questionnaire measuring confidence, knowledge and skill.

Methods: A multidisciplinary team of junior and senior clinicians and transfusion practitioners developed a simulation scenario (which included a debrief) of a patient with TACO and a questionnaire to assess junior doctor's knowledge, confidence and skill level in the management of acute transfusion reactions. A Likert scale questionnaire was developed; with responses ranging from strongly disagree to strongly agree, inclusive of a neutral option (neither agree/disagree). The questionnaire consisted of 15 questions to assess confidence, knowledge and skill .The questionnaire was sent to 40 foundation year 1 and 2 (FY1 and FY2) doctors pre-simulation and again post-simulation. An unpaired T-test was used to determine the differences between the pre and post responses.

Results: Out of the 40 questionnaires sent, 38 participant responses were received pre-simulation, 2 responses were excluded due to an incomplete questionnaire. 22 participant responses were received post-simulation. The post-simulation group showed significant improvement in confidence, knowledge and skills in the management of acute transfusion reactions, as compared with the pre simulation group (Mean, (SD) pre-simulation 2.81 (1.04), post simulation 3.79 (0.71) $P < 0.0001$).

Summary/Conclusions: SHOT UK has highlighted that there is notable increase in TACO cases resulting in major morbidity and death. There is still likely to be underreporting with a need to improve education and training amongst junior doctors. This study has demonstrated that simulation training improved doctors' knowledge, confidence and skill in the management of acute pulmonary complications of transfusion. Following this study, we will develop further simulation based teaching for junior doctors and also assess the impact in the investigation and management of various types of transfusion reactions.

P-029

REVOLUTIONISING TRAINING AT THE NBTC (NATIONAL BLOOD TRANSFUSION CENTRE), CAIRO, EGYPT: AUTOMATION AT ITS BEST

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Background: Automation is important to achieve valid archiving and is an invaluable step towards accurately assessing training. Employees at the NBTC are divided into 7 cadres, and are required to have attained all Basic Training Requirements within 1 year, Intermediate Requirements within 2 years and Advanced from 2 years onwards. The Basic Training Requirement Bundle (BTR) encompasses 5 obligatory courses: Bench Training, Infection Control, Safety and Occupational Health, QMT (Quality Management Training) and the Basic level of Departmental Training.

Compliance to this training bundle is a good measure of the adequacy of training and by extension the competency of working staff in addition to: quality control of the courses, trainee feedback and annual staff competency tests.

Aims: A quantitative study illustrating how automation and shifting from hard copy to digital is a vital step towards accurate training documentation, assessment, and facilitates future planning and customisation of training according to gap analysis.

Methods: A database was created using training tables and rosters for the period from 2000 to 2016, and recorded on an Excel® spreadsheet .Tabulated Data was then imported into a homemade Access® programme where it was manipulated and a formal Training and Scientific Activities Form was generated using the employee identifier and coded by the Quality Department as F/NBTS/TRD/003/02. This relational database has the ability to maintain relationships between data tables and makes it possible to 'connect' data in many ways and ensures data consistency.

Once analyzed, this database provides feedback, facilitates the planning of future training courses, and appraises compliance in target areas that need attention

Results: The database comprised 3,923 records for the NBTC alone, covering 462 employees, and a total of 269 different courses. Analysis of cadres found that Administrative Staff are the most trained numbering 212 employees, with 39.5% of staff trained; followed by Doctors 51, 16.2%; then Lab Technicians 66, 14%;Nurses 57,12.2%;Pharmacists 44, 8.1%;Engineers 16, 5.6%;Chemists 16, 3.9%. Total employees 462, 99.5% trained, with Porters and Recruiters make up the missing 0.5%.

Table (1) Compliance to the BTR components.

Course	No of Staff trained	Compliance Percentage
Safety and Occupational Health	442	95.7%
Bench Training	318	68.8%
Infection Control	206	44.6%
(QMT) Quality Management Training	226	48.9%
Basic level of Departmental Training	201	43.5% compliant

Summary/Conclusions: The success of this project was a testament to the importance of documentation and long term archiving, and that evaluation of training is only possible with proper tabulation, and categorisation into bundles. Compliance to staff training is additionally vital to achieve staff competency. Finally automation geared by creativity in low income settings ultimately allows for resource re-allocation and prudent channelling of training via gap analysis towards the cadres in need of training and courses in need of implementation.

P-030

AN EFFECTIVE ENVIRONMENTAL HYGIENE MONITORING SYSTEM IN DUBAI BLOOD DONATION CENTER. IS SEEING TRULY BELIEVING?

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Background: Ensuring a good standard of cleanliness and hygiene in Dubai Blood Donation Center (DBDC) is always observed and maintained following consistent and correct cleaning procedures. But is this method enough to truly believe that the facility is considered to be a healthy and safe environment for the employees and its customers? Since there is an "evolving mandate" that environmental hygiene must be objectively analyzed and optimized, the facility has adopted an environmental monitoring tool to assess the effectiveness of the cleaning procedure that started in 2014.

Aims: The aim of the study is to assess effectively the cleanliness of the environmental surfaces of the facility in real time. Surfaces, although appearing visibly clean, can still harbor significantly high levels of contamination.

Methods: The facility used the clean trace system that measures the levels of adenosine triphosphate (ATP), an excellent marker for organic contamination or contamination from biological source, on an environmental surface. A clean trace surface test is used to swab the frequently touched items (test points) such as equipment, machines, medical items, etc. every 2 months. The clean trace surface test is then placed in the Luminometer machine to measure the level of ATP and produces a result expressed in Relative Light Unit(RLU).The test is performed in less than 30 seconds, providing a real time result. The DBDC has set a pass/fail threshold values with the acceptable range of 0-150RLU. The higher the RLU level, the greater the risk it poses to encourage growth of microorganisms and possible cross contamination which can lead to infection and contamination. If the result is >150RLU, a corrective action is taken such as coordinating with the concerned Unit to perform immediate cleaning and disinfection of the affected item. In cases where a re-clean and retest approach does not address the problem, the following steps in the cleaning process are evaluated. The facility has formulated an Infection Control Committee who conducts training to the staffs. Our continuous improvement steps is the ongoing review of data to understand whether the process of routine monitoring and corrective action has led to reduced levels of contamination either at the test point or Unit level.

Results: In 2014, an average of 41% of the test points have failed showing high levels of RLU. In 2015, it has decreased to 22% and last year, it dropped to 16% showing an overall decline in the level of contamination. This is a strong evidence that the RLU levels reduced over the time of assessment.

Summary/Conclusions: It has brought the facility a major step in hygiene monitoring. Healthcare workers including the customers of DBDC are protected from risks of infection and contamination that is associated with healthcare facilities, environment or contaminated surfaces. Our continuous improvement process allows us to review and maintain improved standards of cleanliness. It has also introduced a good spirit of competition among staffs to ensure their Units are the cleanest.

P-031

DEVELOPMENT OF ORIENTATION PROGRAMME (FOR NEW STAFF) IN BLOOD DONATION AS PART OF KNOWLEDGE MANAGEMENT

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Background: The Finnish Red Cross Blood Service's (FRC BS) orientation programme for new staff has been in use in Blood Donation since 2012. The programme is built on an online platform and it contains modules for both general training on blood donation operation and for specific training on tasks, processes and requirements at work. The supervisor and the new employee together choose one task at a time to qualify for. The length of the orientation is usually 2 to 3 weeks.

Aims: The aim is to utilize the results of a thesis done by an FRC BS employee in developing the orientation of the blood donation operation by using existing orientation material and paying attention to different learners.

Methods: The data was collected from an online questionnaire which was sent to new or returning employees who were in orientation during 2014-2015 and were still working in blood donation operation (n = 58). The data was analyzed by utilizing descriptive statistical methods.

Results: The questionnaire was sent in March 2016 to 58 new or returning employees and 30 (52%) of them answered the questionnaire. 43% (n = 13/30) were new employees and 57% (n = 17/30) were returning employees.

The results showed that all employees were mainly satisfied with the implementation of the orientation. Enough time was allocated for the orientation. The content of the orientation material was evaluated to be mainly clear (90%, n = 27) and it supported the orientation (96%, n = 29).

However, there had also been some challenges with how to use the material (53%, n = 16). These were mainly caused by the uncertainty of utilization and timing of the orientation material and also the copious reading of the directives. Most of the employees (87%, n = 26) learn new things mostly by doing, trying and practicing. 84% (n = 25) partially agreed or disagreed that the learning styles were taken into account. Only 31% (n = 4/13) of the new employees completely agreed that the previous know-how of the employee was taken into account.

Summary/Conclusions: As a conclusion the orientation of blood donation operation is implemented by experiential learning process whereby knowledge is created through the transformation of experience. The orientation is planned together with the new employee and the previous know-how of the employee is taken into account. During the orientation the different learning methods are utilized widely.

The orientation is getting a more visible part in the knowledge management of the organization utilizing the profiles of know-how. The new employee discusses with the supervisor, utilizing these profiles, how the orientation has gone and how to develop the know-how further.

To standardize orientation and make the material and its content easy to understand and the progress easy to monitor, the structure and content of the orientation material are described in a visual form. The roles of those taking part in the orientation are also described visually in a way that is easy to understand.

P-032

Abstract has been withdrawn.

P-033

PATIENT BLOOD MANAGEMENT AND THE FORMATION OF MEDICAL COMPETENCE IN THE MEDICAL SIMULATION CENTER OF BOTKIN HOSPITAL

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Background: Among the most meaningful development vectors of modern medicine it is necessary to emphasize the following ones: efficiency improvement, new technologies, targeting and minimizing the impacts and complications resulted from medical treatment. The main problems of transfusion medicine are the cost of technologies and the risk of impact on patients. The contradictions are unveiled in such issues as standardized/individual approach, for doctors - having a narrow specialization/being multidisciplinary, use of new technologies/deficiency of knowledge and the need for trainings. The development of medical insurance case study has led to increasing demand for intensive and multidisciplinary medical treatment programs. With the multidisciplinary approach in optimizing the transfusion practice Patient blood management allows improving both medical and financial values.

Aims: The successful performance of Patient blood management concept is possible when the formation of medical competence aims to establishing well-coordinated logistics relationship: administrator – transfusiologist; clinician – transfusiologist; doctor – nurse; doctor – patient.

Methods: For medical specialists who practice blood component transfusion at Medical Simulation Centre of Botkin Hospital there is a two-day (18 academic hours) intensive multi-field training course. The number of trainees is up to 35 in a group. The course is divided into 6 lectures and also gives the learners the opportunity to participate in 3 seminars, 2 interactive clinical discussions, a master-class including the modeling workflow scenarios.

Results: The medical competences gained:

- Interpret the modern parameters of the hemogram, coagulogram and thromboelastogram.
- Reveal the indications and contraindications for urgent and scheduled blood component transfusion.
- Plan the necessary transfusions.
- Personalize the blood transfusion risks.
- Have an individual approach in choosing the type and the amount of transfusion habitat.
- Foreseen the impact of transfusions.
- Correct the anemia and hemostasis system malfunctions using medicinal treatment.
- Carry out the macroscopic assessment of hemo-transfusion habitats before the procedure.
- Use the differentiated diagnostics and prescribe the transfusion complication treatment.
- Evaluate the result of each transfusion.
- Carry out the auditorial check of health cards.

In total, about 200 doctors from hospitals in Moscow were trained in this program.

Summary/Conclusions: The additional professional training program for healthcare professionals was implementation. Programm was named «Topical issues of safety and efficiency of using blood components in adult patients; the legal regulation, risks and alternatives of blood transfusion in different hospitals». This program to meet the educational and professional demands of specialists, development of algorithms of thinking for patient blood management. It helps adapt clinician qualifications to the changing conditions of professional activity and the social environment.

Risk models, standards and regulation

P-034

THE BLOOD DROUGHT IN INDIA: DEFINING TIMELY ACCESS AND OVERALL DONOR SAFETY TO INFORM MODELLING OF AN EFFECTIVE SCALE-UP

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Background: Access to safe, timely, and affordable blood transfusion is integral to the provision of care in low and middle income countries (LMICs). In India, there is a shortfall of approximately 3 million units of blood each year. It is believed that the illegal practice of unbanked direct, whole blood transfusion (UDBT) continues. Physicians cite lack of timely access and cost as reasons for this. Recent donor-driven efforts in many LMICs have focused on the development of centralized blood banks. This has reduced the spread of transfusion transmittable infections (TTI's), but has also increased costs, sometimes raising the price of a single unit above annual per capita health expenditure. In other LMIC settings, decentralized systems and UDBT have been shown cost-effective. UDBT often relies on replacement donors. These donors are thought to carry higher risk of TTI, though several recent studies have contested this.

Aims: 1) Define the extent of untimely access to blood in India and the frequency of UDBT; 2) Evaluate TTI prevalence in general, replacement, and voluntary Indian donors; 3) Develop a cost-effectiveness model utilizing the context of post-cesarean bleed to evaluate total health system costs for four systems: hospital-based banking; community banking; UDBT as currently practiced; and idealized UDBT.

Methods: To assess timeliness, we relied on expert opinion. We conducted a 21-question multiple choice convenience sample survey of 39 Indian surgeons attending a conference. Hospital size ranged from 12-290 beds; 8/29 states were represented; 14 respondents reported in-facility banks. To inform likelihood of mortality and sequelae following untimely transfusion we will also rely on expert opinion. To define TTI rates in replacement, voluntary, and general donors, we utilized the literature, using studies from only the last 10 years utilizing ELISA. A PubMed search returned 28 titles. Seven articles were reviewed and back-referenced to find relevant non-Medline-indexed studies. A total of 14 articles from 7 states met criteria. The combined sample included 620,431 donors. To inform our cost-effectiveness model, we have relied on data from India, including: probability of mortality due to bleed following cesarean, chronic disease burden, chronic disease treatment costs, equipment costs, and test failure rates. We have relied on data from other LMICs for mortality in those unable to access transfusion following cesarean.

Results: Respondents reported that blood is available 63.8%. In-house banks improved likelihood of availability (71.4% vs. 59.6%). Of those with banks, 53.8% reported being able to transfuse within 30 min. Of those with community blood banks, 12.5% could do so. When unable to transfuse, 56.4% reported performing UDBT. Amongst all blood donors, HIV, HBV, HCV, and VDRL occur at 0.26%, 1.18%, 0.36%, and 0.18% respectively. Rates were notably higher among replacement donors as compared to voluntary donors (HIV 0.20% vs. 0.12%, HBV 1.44% vs. 0.66%, HCV 0.48% vs. 0.12%). Results of cost-effectiveness analysis are pending data on untimely transfusion.

Summary/Conclusions: Blood shortage in India remains severe. Timely access is limited for facilities both with and without banks. The use of UDBT is widespread. TTI's remain prevalent among donors, though notably less so among voluntary ones. This data is consistent with general knowledge regarding donor selection but conflicts with recent studies from several other LMICs. Conclusions surrounding cost-effectiveness are forthcoming.

P-035

MAXIMAL BLOOD ORDER SCHEDULE (MSBOS): DEFINITION OF INSTITUTION TRANSFUSION RISK BASED ON THE ANALYSIS OF THE ELECTIVE SURGICAL PROCEDURES CODED IN THE HOSPITAL DISCHARGE FORM

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Background: The definition of the local Maximal Blood Order Schedule (MSBOS) is a required item in the Quality Assurance of medical records controlled by the local Health Authorities in Italy. Institution specific version of the MSBOS can lead to better transfusion practices.
Aims: Primary aim of the study was to rate the risk of blood transfusion related to elective surgery in our hospital using routinely collected data. Secondary aim was to create a standardized benchmarking system of blood transfusion in surgical departments which was reproducible and comparable with other healthcare facilities.
Methods: Routinely collected data are increasingly being used to describe and evaluate transfusion practice. We performed a retrospective analysis of the data coded in the Hospital Discharge Form (HDF) (79619 HDFs) and the transfusion data recorded (51288 packed RBCs) on a dedicated database (Emonet, Insiel, It) during the period 2014-2016 (first semester). Data were included if the hospitalisation was scheduled and the admission was in a surgical Department; ICD-9 codes of surgical procedures in the HDF were linked through the patient's social security number to transfusion data in Emonet only if the transfusion took place the same day or the day after the surgery. The risk of blood transfusion related to each elective surgical procedure was therefore calculated as a rate and the consumption of units of blood was described with a Box Plot showing the outliers. Results of a pilot analysis, limited to the cardiac surgery department, are presented below; the analysis of the other surgical departments is ongoing.
Results: Results of a pilot analysis, limited to the cardiac surgery department, are presented below; the analysis of the other surgical departments is ongoing. As an example, in cardiac surgery the total risk of transfusion for the procedures code 36.1 - "Bypass anastomosis for heart revascularization" was 89% (383 procedures related to a transfusion out of 432 codified procedures during the reporting period) with an average consumption of 2,9 (1-14) units of blood and a median of 2; the analysis was also stratified using child code below 36.1 with greater detail. With a multidisciplinary approach, a team of experts used these results as an additional criteria (beside the available evidence, the clinical and surgical experience, the logistic model of transfusion in our Organisation) to redefine at 1 unit the MSBOS value for this type of procedure. The same evaluation was extended to all the surgical procedures performed by cardiac surgeons.
Summary/Conclusions: The analysis of routinely collected data in HDF and Emonet database may be successfully used to evaluate and monitor over time the risk of transfusion and the consumption of blood units related to scheduled surgery in order to define and keep updated the local standard of MSBOS values. In the future, the descriptive statistical analysis of blood consumption will allow the identification of outliers which may be candidate to a clinical audit in order to improve the quality of services; moreover, this method allows to record the trend of the transfusion practice in the hospital and can be used as a benchmarking system between healthcare facilities.

P-036

IN BLOOD TRANSFUSION ENVIRONMENT, WHY BIOSAFETY IS SUCH AN IMPORTANT ISSUE

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Background: We are living in an era of uncertainty and change. New infectious agents and diseases have emerged. Work with infectious agents in public and private research, public health, clinical and diagnostic laboratories, and in animal care facilities has expanded.
Aims: For these reasons, organizations and laboratory directors are compelled to evaluate and ensure the effectiveness of their biosafety programs, the proficiency of their workers, as well as the capability of equipment, facilities, and management practices to provide containment and security of microbiological agents.
Methods: A fundamental objective of any biosafety program is the containment of potentially harmful biological agents. The term "containment" is used in describing safe methods, facilities and equipment for managing infectious materials in the laboratory environment where they are being handled or maintained. The purpose of containment is to reduce or eliminate exposure of laboratory workers, other persons, and the outside environment to potentially hazardous agents.
Results: Published reports of LAIs first appeared around the start of the twentieth century. By 1978, four studies by Pike and Sulkin collectively identified 4,079 LAIs resulting in 168 deaths occurring between 1930 and 1978.
The authors acknowledged that the 4,079 cases did not represent all LAIs that occurred during this period since many laboratories chose not to report overt cases or conduct surveillance programs to identify sub-clinical or asymptomatic infections.
Summary/Conclusions: In conclusion, investigations of LAIs have identified five principal routes of laboratory transmission. These are parenteral inoculations with syringe needles or other contaminated sharps, spills and splashes onto skin and mucous membranes, ingestion through mouth pipetting, animal bites and scratches, and inhalation exposures to infectious aerosols.

Blood supply management and utilization

P-037

WHO IS USING BLOOD AND WHEN - IMPACT OF THE AGEING POPULATION ON DEMAND FOR AND URGENCY OF TRANSFUSION

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Background: Previous studies report that patients aged 65 years and above consume 50% or more of the blood supply. It has been reported that growth in the older population will place pressure on blood supplies.
Key Patient Blood Management (PBM) strategies include optimisation of patient's physiology prior to interventions that may require transfusion support. Patients transfused soon after Emergency Department (ED) presentation are more likely to have deranged physiology, with limited or no opportunity for optimisation of anaemia status, or modification of anticoagulant medications. This challenges PBM based approaches to blood management.
In addition, our recent review of evidence relating to transfusion outcomes in older adults identified that liberal rather than restrictive transfusion strategies may have better outcomes in this cohort. This has implications for overall red cell use.
Aims: To explore blood use in older patients, with a focus on ED presentations. Blood use was stratified by age, gender, urgency and indication to gain insight into rates and reasons for transfusion in the older population. We sought to understand blood use in the context of regional population demographics.
Methods: Data was obtained for 5,294 blood products transfused over a 12-month period in an Australian regional health and hospital service. Blood data was linked to 85,014 ED presentations. The age and gender of each recipient was obtained via linkage to the hospitals' patient admissions systems. The number of blood products transfused within 24 h of ED presentation was quantified, and separated from non-ED related blood use. The patient admission and ED information systems were interrogated to obtain information including the reasons for presentation and discharge diagnoses. Overall population analysis was undertaken to identify ageing trends in the region under study.
Results: The proportion of the population aged 65 years and above in the study region was 19.8%, which is 30% higher than the Australian national average. The rate of ED presentations per 1,000 population increased significantly with age, with higher rates for older males than females.
Sixty percent of all blood products were transfused to patients aged 65 years and above, which is higher than reported in other studies. The pattern of red cell use per 1,000 population stratified by age mirrored data published for England and North Wales.
28% of blood products were transfused to patients within 24 h of ED presentation, indicating a significant level of unplanned transfusion.
For those aged 65 years and above, females consumed three-fold and males greater than five-fold more red cells per 1,000 ED presentations than patients below 65 years of age.
Summary/Conclusions: In this study, blood consumption by those aged 65 years and above is higher than previously reported, and strongly skewed towards males. In the

context of an ageing population and with male life expectancy increasing relative to female, these findings have significant implications for future supply and demand.

P-038

PHENOTYPED RED CELLS – MEETING INCREASED DEMAND

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Background: In 2015 the Australian Red Cross Blood Service introduced a nationally standardised process to facilitate improved phenotyped inventory, age of issue and order fulfilment. A "pull' system with testing requirements determined based on actual inventory levels and the age of the inventory was established.
Aims: Since 2006 all new donors have been tested for extended Rh (C, c, E & e) and K and historically group O and A whole blood donors that are R_1R_1, R_2R_2 and rr are selected for further phenotyping for Fy^a, Fy^b, Jk^a, Jk^b, M, S and s.
Methods: A reporting tool has been developed to measure DIFOT (delivery in full and on time) for orders of red cells that have specific phenotype requirements. The percentage of phenotyped orders delivered in full and on-time is available nationally and by region for the day, month and financial year. Detailed information for each order can be accessed through the report to investigate and identify opportunities to improve supply.
Results: Since June 2015 the Blood Service has achieved greater than 97% DIFOT each for phenotyped blood requests, with exception of 5 out of 19 months where DIFOT dropped only as low as 95.1%. These months coincided with either reduced whole blood inventory, increased phenotyping requests or reduced testing capacity. In addition the Blood Service has identified that a significant number of failures are due to ABO and RhD blood group substitution of red cell products. In a number of cases RhD negative red cell components are being provided to patients that are RhD positive to meet phenotype requests, particularly for patients with the R_oR phenotype, adding to the increased demand for RhD negative, rr red cells.
Summary/Conclusions: The Blood Service has expanded phenotyping to include R_oR to alleviate some of the unnecessary demand for RhD negative, rr red cells. Retrospective testing for extended Rh (C, c, E & e) and K of donors enrolled prior to 2006 is underway to increase supply of inventory with extended Rh and K phenotyping and routine blood group phenotyping will be transferred to an automated testing platform to increase the volume of testing. These actions are designed to ensure the Blood Service keeps pace with increasing demand, enabling greater access to phenotyped including blood groups other than group O and A and ensure appropriate use of RhD negative red cells.

P-039

RFID-BASED TECHNOLOGY TO IMPROVE BLOOD SUPPLY MANAGEMENT IN PERIPHERAL HOSPITALS

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Background: Our regional blood centre is brought to provide a swift and lossless supply of blood products to a 30 km away hospital for its elective orthopaedic and general surgical activity. Anaesthesiologists claim that nominative Red Blood Concentrates (RBC) should be available before starting their procedures. The demand vs. use ratio was 2:1 in this context.
Aims: In compliance with PIC/S GMP guidelines, we needed to improve the efficiency of the inventory management and supply chain. The goal was to ensure the availability of nominative blood products and their on-time delivery while reducing the risk of wastage by implementing a safe return of unused products.
Methods: The Biolog-id solution is a global "Tracking Management System" based on RFID technology that uses an electronic label (an HF 13.56 MHz passive RFID tag) which contains all the key information about the blood unit including, for nominative RBC's, all the information concerning the patient to whom the product is intended.
The RFID tag allows a contactless high speed read/write as well as proprietary encryption for data protection. The electronic label is associated with a Smart Storage RFID kit (SST-R) designed to be integrated into medical refrigerators or cold rooms. The SST-R allows a real-time localization of blood units, temperature monitoring and remote allocation. The system automatically traces and records any movement that can be then linked to a determined cause allowing the identification of any blood products whose storage conditions are not satisfactory to prevent their use.
The deployment of this RFID based solution, interfaced with our own IT system, allows us to manage and track shipments between our centre and the hospital while recording the storage temperature on a real time basis.
It also permits a remote management of the inventory of blood products at the hospital with a real time visibility of the stored products (product, phenotypes, validity etc.), directly from our Blood Centre. The system also enables nominative delivery at distance.
Results: The implementation of this system resulted in a substantial improvement of blood supply management at the target hospital, by significantly reducing the number of claimed but unused blood products.
Before using the system, the ratio of returned/discarded products was 62% vs only 38% effectively transfused. From July 2014 to July 2016, this ratio was reversed: 61% of transfused products with a drop of returned products down to 39%. Furthermore, transport, storage and return conditions now being highly controlled, the returned RBC's can safely be affected elsewhere.
Summary/Conclusions: Our Blood Center is now able to efficiently monitor and manage distant hospitals' blood inventory. This directly leads to an improvement of patient safety by ensuring a steady supply of available blood products directly on site. The Biolog-id solution also helps in minimizing unnecessary movement of blood products through remote reallocation and the ability to anticipate need in blood supply. It also allows an automated and global traceability for our products.

P-040

AVAILABILITY AND SAFETY OF BLOOD TRANSFUSION DURING HUMANITARIAN EMERGENCIES

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Background: Risks to health caused by humanitarian emergencies are a major concern in the Eastern Mediterranean Region of the World Health Organization (WHO). There are more than 76 million people affected by humanitarian emergencies, including almost 16 million refugees and 10 million internally displaced people in the Region, where some of the most affected countries in the world are located. In these countries, the health systems have been weakened or destroyed and health workers provide health services in insecure and under difficult circumstances. Humanitarian emergencies increase the demand for blood transfusion and make its delivery challenging and complex. Despite these obvious needs, across the Region, there is a lack of information on the emergency preparedness and response capacity of blood transfusion and on the challenges countries and health responder's face in meeting the needs of the patients during emergencies.
Aims: Assess the blood transfusion availability and safety during humanitarian emergencies in the Region.
Methods: We searched PubMed and Index Medicus for the WHO Eastern Mediterranean Region for data on availability and safety of blood transfusion during humanitarian emergencies. We conducted a structured survey of blood transfusion services (BTS) in all countries in the Region to identify the following: type of humanitarian emergencies between 2006 and 2016; current strategies to ensure availability and safety of blood transfusion during emergencies; coordination and collaboration between countries; and gaps and challenges. Additional information was collected during a regional consultation on the availability and safety of blood transfusion during humanitarian emergencies held in May 2016 in Tunisia.
Results: We found 24 publications on disaster from five countries in the Region and 16 publications on disaster preparedness and blood transfusion in casualties and severe trauma outside the Region. However, none dealt with the questions of availability and safety of blood transfusion during humanitarian emergencies. Twelve countries (54.5%) responded to the survey. Armed conflicts and terrorism, flooding and earthquakes are the commonest types of emergencies with estimated 10-85% of the injured requiring blood transfusion. Nine countries have emergency preparedness and response plans to meet increased demand for blood transfusion. Potential blood donors are mobilized through public calls, besides a direct appeal on regular and family/replacement donors. Seven of the responding countries keep an emergency blood stock.

Collaboration between the different medical and emergency care providers exists in seven countries. Lack of adequate and competent human resource, transport and cold chain deficits, shortages in supply of consumables and maintenance of equipment, lack of reliable power supply, and shortage in finances are the gaps identified.

Summary/Conclusions: There is a need to integrate BTS in the overall national emergency preparedness and response, collect and disseminate updated information on factors affecting provision of blood transfusion during humanitarian emergencies, provide technical and financial assistance to affected countries, strengthen mechanisms for coordination and collaboration among different parties, and develop a regional emergency blood services system and management expertise; recommendations for individual countries need a tailor-made basis to guide and advise, along the lines of the regional strategic framework for blood safety and availability.

P-041

A FIRST IN THE COUNTRY: AN ASSESSMENT OF PLATELET USE IN A PHILIPPINE TERTIARY PEDIATRIC HOSPITAL

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Background: Platelet component therapy, which can by life-saving, is also capable of doing harm, especially when used unnecessarily. It is responsible for majority of blood component transfusion reactions, carrying with it the risk of transfusion transmitted infections, febrile and allergic reactions, alloimmunomodulation, and platelet refractoriness. Also, platelets are not a ready commodity especially in a developing country where the demand usually outweighs the supply.

As such, there is a need to ensure appropriate use of this valuable resource to avoid unnecessary harm to the patient and to avoid wastage.

Aims: To investigate if the current hospital practice for transfusions of platelets in the Philippine Children's Medical Center conforms with the institution's current transfusion guidelines.

Methods: A 3-month retrospective platelet audit was carried out at our tertiary care 200-bed hospital housing various specialties but devoted to hemato-oncology. All patients below 18 years of age who received platelet transfusions in any of the pediatric sections- including the general pediatric service, both the pediatric and neonatal intensive care units, the pediatric hematology section, the pediatric neurology section, and the pediatric surgery unit- were included in the study. Each transfusion episode was assessed whether it satisfied the predetermined criteria set by the home institution, which references manuals from both the Philippine Department of Health and the American Association of Blood Banks.

Results: From July to September 2015, approximately 1,854 units of platelets [Random Donor Platelet or RDP, 1821 (98%); and Single Donor Platelet or SDP, 33 (0.2%)] were prepared and transfused to 201 patients (RDP to 192 patients and SDP to 9 patients) in 652 transfusion episodes.

Hematology-oncology was the main user specialty utilizing 90.9% of the units prepared, followed by the pediatric and neonatal intensive care units (6.1% and 2.8%, respectively). Eighty-seven percent of platelet transfusions were appropriate as per the recommended institution guidelines. However, 13% of the prophylactic platelets were transfused inappropriately. The most commonly used indication for transfusion was "Prophylaxis for severe thrombocytopenia <20,000/l or associated platelet defect"- this was also the most abused reason to justify unwarranted transfusions. Ninety-five percent of inappropriate episodes were from the haematology-oncology unit, followed by the neurology section (2.5%), and the pediatric intensive care unit (2.5%).

Summary/Conclusions: While based on literature review, our hospital's compliance to platelet transfusion guidelines of 87% is very well within normal limits, there should be a zero tolerance policy when it comes to unwarranted blood transfusion interventions.

Ultimately, the goal of audits is to reduce inappropriate transfusions in patients and identify areas of further improvement.

P-042

BLOOD INVENTORY LEVELS IN POLISH BLOOD TRANSFUSION SERVICE – ANALYSIS OF SEASONAL VARIABILITY (2014–2016)

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Background: The capacity of blood transfusion service to provide adequate supplies of blood components is the issue of concern for health providers and health service worldwide. In Poland the annual rate for red blood cells (RBC) transfusions is over 1 million units and for the most part the 23 Polish Blood Transfusion Centers are well able to meet this demand. In certain circumstances however the inventories prove insufficient to meet the current clinical needs and restocking becomes a necessity.

Aims: The aim was analysis of blood inventory variability in the period 2014–2016, with special emphasis on identification of those periods when blood shortages become apparent.

Methods: Retrospective analysis of 2014–2016 data related to blood inventories in Polish Blood Transfusion Centers. In the consecutive months of the 2014–2016 period the average daily inventory of RBCs was evaluated as expressed by day's cover i.e. available stock to cover daily clinical needs (based on up to date consumption).

Results: The average daily national inventory of RBC (all ABO groups) expressed by day's cover is as follows:

- 2014 – 3.55 to 9.43 day's cover (5.91 on average).
- 2015 – 4.38 to 7.25 day's cover (5.78 on average).
- 2016 – 3.35 to 8.46 day's cover (5.47 on average).

Differences in average blood inventory levels revealed specific seasonal variability:

- In January and February each year the RBC inventory supply was sufficient for 5.77–6.28 day's cover;
- In March and April each year the RBC inventory supply was sufficient for 7.07–9.43 day's cover (highest values);
- In May a decreasing tendency was observed with lowest inventories in July (2015 – 4.38 days) and August (2014 – 3.55, 2016 – 3.35 day's cover); urgent appeals for blood donation were then frequently broadcasted;
- In autumn the inventories were lower as compared to the beginning of the year and spring, with the lowest values in November (3.96–4.75 day's cover);
- In December, the inventory levels increased and the RBC inventory was usually 5.14–7.25 day's cover.

The pattern repeated for each of the years in the analyzed period. The presented data are mostly of indicative value. The inventory levels in individual Blood Transfusion Centers may widely differ from the national average. Moreover, local RBC shortages by ABO groups were also reported.

Summary/Conclusions: A 5- to 7-day's cover seems to be the desirable goal for Polish Blood Transfusion Centers although a 3 day's supply may serve as adequate "safety threshold". While blood stock depletion in the summertime is observed in Poland as well as in many other countries and found mainly related to holiday absence of blood donors, the reported low blood supply in November with subsequent increase in December (i.e. during winter holidays) is not a typical phenomenon and definitely merits further analyses.

P-043

INTRODUCTION OF COMPLIANCE TRANSFUSION AUDIT IN A TERTIARY CARE HOSPITAL BASED BLOOD BANK AS A TOOL FOR GOOD CLINICAL TRANSFUSION PROCESS

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Background: Audits are essential aspect of blood transfusion process & as a management tool in blood transfusion. Various types of audits can be envisaged in blood transfusion – operational, quality process, competence, compliance, so on. Every hospital/blood centre is expected to develop a system of audit, appropriate to its needs. We, in our hospital, started the process of auditing compliance of guidelines for few parameters in transfusion process.

Aims: To improve transfusion process compliance against set guidelines & SOPs, thus to ensure better patient blood management & safer outcome.

Methods: Two & half year data from July 2014 to December 2016 has been collated. All blood products– pRBCs, RDP, SDP & FFP, from issue to transfusion were followed for compliance. Audit performa was prepared for bedside evaluation of transfusion record sheet of the patient for blood products issued in last 24 h. Parameters audited from Transfusion Record Sheet (TRS) were–transfusion start delay >1/

2 h, transfusion start and end time, compatibility label, signature of doctor & staff nurse in TRS, Transfusion Reaction Form (TRF) status-eventful/uneventful & return of empty bag for autoclaving. Daily & monthly data was compiled and evaluated for noncompliant parameters. Quarterly data presented in Hospital Transfusion committee (HTC) for assessment of areas (wards/OTs) of high noncompliance and inputs for improvement. Steps undertaken for improvement- classroom training of the Staff nurses and resident doctors, worksite guidance by auditor, involvement of management & senior doctors through HTC. Effect of audits has been analysed for period of six months regularly.

Results: Blood products issued in Jan-Dec'14-4576, Jan-Jun'15-5755, July-Dec'15-6494, Jan-Jun'16- 4905 and July-Dec'16-6072. Data not available in-1) **Start time** 3.97% (July-Dec'14), 1.77% (Jan-Jun'15), 0.52% (July-Dec'15), 1.14% (Jan-Jun'16) and 1.77% (July-Dec'16).2) **End time** 12.6% (July-Dec' 14), 6.62% (Jan-Jun'15), 2.14% (July-Dec'15), 6.76% (Jan-Jun'16), 5.05% (July-Dec'16). 3) **Product label** 1.3% (July-Dec'14), 0.46% (Jan-Jun'15), 0.06% (July-Dec'15), 0.3% (Jan-Jun'16), 0.29% (July-Dec'16) 4) **Doctor sign on TRS** 9.1% (July-Dec'14), 6.34% (Jan-Jun'15),0.93% (July-Dec'15), 4.91% (Jan-Jun'16), 3.16% (July-Dec'16). 5) **S/N sign on TRS**– 3.2% (July-Dec'14), 1.28% (Jan-Jun'15), 0.55% (July-Dec'15), 0.85% (Jan-Jun'16), 0.83% (July-Dec'16). 6) **TRF** 3.3% (July-Dec'14), 0.38% (Jan-Jun'15), 0.04% (July-Dec'15), 0.02% (Jan-Jun'16), 0.0% (July-Dec'16). 7) **Emptybag** 5.6% (July-Dec'14), 0.34% (Jan-Jun'15), 0.06% (July-Dec'15), 0.02% (Jan-Jun'16), 0.0% (July-Dec'16).8) **Eventful** 0.06% (July-Dec'14), 0.36% (Jan-Jun'15), 0.26% (July-Dec'15), 0.12% (Jan-Jun'16), 0.098% (July-Dec'16).9) **Uneventful** 99.9% (July-Dec'14), 99.6% (Jan-Jun'15), 99.7% (July-Dec'15), 99.8% (Jan-Jun'16), 99.9% (July-Dec'16).10) **Transfusion started>½hour**23% (July-Dec'14), 18.7% (Jan-Jun'15), 13.1% (July-Dec'15), 15% (Jan-Jun'16), 11.6% (July-Dec'16).

Summary/Conclusions: Significant sustained continuous improvement in all parameters was seen which proved the effectiveness of regular compliance audits. Sudden transient downfall found from Jan-Jun 2016 in few parameters which was studied and observed to be attributed to multiple factors: high attrition of nurses and resident doctors, high patient turnover with increased nurse:patient ratio, training missed by new nurses & residents due to workload, etc. Strict monitoring and documentation of the blood transfusion strengthens our handling of clinical, medicolegal & quality implications. Also regular transfusion audits and training improve clinical transfusion process and patient outcome with strengthened transfusion reaction reporting.

P-044

BLOOD ORDERING AND TRANSFUSION PRACTICES OF UROLOGY DEPARTMENT IN A TERTIARY HOSPITAL DURING A 5-YEAR PERIOD

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Background: Appropriate transfusion practices ensure patient safety and prevent blood shortages and increased costs. Throughout the last 5 years, the Department of Transfusion Medicine and Blood Bank from Centro Hospitalar São João, EPE, has been focusing in education of clinicians, as well as promotion and introduction of several strategies to reduce unnecessary blood ordering and transfusion.

Aims: Evaluate blood ordering and transfusion practices in the Urology department before and after the implementation of MSBOS (Maximum surgical blood order Schedule) and restrictive red cell transfusion strategy (single unit policy and restrictive haemoglobin thresholds).

Methods: The data were collected from our blood bank database and from official activity reports of our institution from the years 2011 till 2015. Blood-ordering and blood transfusion, number of surgeries and hospital discharges from the Urology Department were reviewed retrospectively.

Results: The rate of red blood cell (RBC) transfusion clearly decreases along the years reviewed (63% decrease), though there was an increase in the number of surgeries (10% increase) and number of patients discharged (28% increase) from the Urology Department. The number of red blood cell (RBC) transfusion during hospitalization in the Urology Department has decreased 61%, (451 RBC in 2011 to 177 in 2015) and 69% during surgery (167 units transfused in 2011 to 52 units in 2015). Before the implementation of MSBOS, in 2011, the crossmatched RBC/transfused RBC (C:T) ratio in urologic surgeries was 9.4. In 2015, the C:T ratio was 2.1. Prior to implementation of the single-unit policy, in 2011, only 20% of all RBC transfusions were single-unit transfusions. By 2015, 70% of all RBC transfusions in Urology were single unit RBC transfusions.

Summary/Conclusions: A downward trend in RBC transfusion rate was observed. Despite the growing number of patients and surgeries through the years, the number of RBC transfusions has decreased. Transfusions are being delivered as single-unit episodes with reassessment after each unit, leading to fewer second units being prescribed. Although MSBOS has brought a huge reduction in the C:T ratio, we aim to achieve a crossmatch-to-transfusion (C/T) ratio of <2.0, consistent with national and international recommendations. Changes in surgical techniques, with evolution to minimally invasive surgery, surely also played a role and had an impact in reducing the need for transfusion in the Urology Department.

P-045

A PROSPECTIVE STUDY OF BLOOD UTILIZATION IN ELECTIVE SURGERIES FOR FORMULATION OF MAXIMUM SURGICAL BLOOD ORDERING SCHEDULE (MSBOS) AT A TERTIARY CARE TEACHING HOSPITAL IN CHANDIGARH, INDIA

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Background: Arbitrary and excessive requisition of blood for elective surgeries is a common practice leading to wastage of blood. To be on the safe side, surgeons request more packed red blood cells (PRBCs) than required for elective surgeries. Maximum Surgical Blood Ordering Schedule (MSBOS) is an evidence based method allowing efficient use of blood stocks, optimal utilization of blood bank resources and reducing blood wastage. This study was undertaken at a tertiary care teaching hospital in India to determine MSBOS for certain elective surgeries. Around 45,000 surgeries are performed in the institute annually.

Aims: To study blood requisition and utilization pattern of nine elective surgical procedures in three surgical departments (Surgery, Orthopedics, and Gynecology) and determine MSBOS.

Methods: All patients undergoing nine elective surgeries -Abdominal Hysterectomy (AH), Vaginal Hysterectomy (VH), Exploratory Laparotomy-Gynecological (ELG), Exploratory Laparotomy-Surgical (ELS), Whipple's procedure (WP), Frey's Procedure (FP), Splenectomy, Total Hip Replacement (THR), and Total Knee Replacement (TKR) – from 1st June 2015 to 31st August 2015 were included in this prospective study. Data of blood requisition, issuance, transfusion, and return within 24 h for each patient was collected prospectively from patient record, operating room and blood bank. Descriptive analysis was performed to determine CT Ratio, Transfusion Probability, Transfusion Index (Ti) and MSBOS for each surgical procedure and altogether. The study was approved by institute review committee.

Results: A total of 536 patients underwent these surgeries during the study period, out of which 67.9% were females. Mean age of patients was 48.6 years (SD 14.8). Total 1380 PRBC units were requested but only 813 (58.9%) were issued by blood bank. Mean PRBC units requested per surgery was 2.57 (max 3.33 for Splenectomy and min 2.06 for VH). Out of 813 units issued, 244 (30%) were transfused to 207 (38.6%) patients. Maximum 51.47% TKR patients received transfusion whereas only 22.2% Splenectomy patients were transfused. Out of 569 units not-transfused, only 297 (52.2%) were returned to blood bank within 24 h. There was no statistically significant difference ($P = 0.3$) in pre-transfusion hemoglobin of patients transfused (6.9 g/dl) and not-transfused (7.1 g/dl). Total 824 cross-match were performed. CT ratio for all surgeries was 4.18 (max 7.4 for Splenectomy, min 2.53 for AH). Overall Transfusion Probability was 35.17% (max 51.47% for TKR, min 22.2% for Splenectomy), and Ti was 0.41 (max 0.62 for TKR, min 0.28 for Splenectomy). The MSBOS for elective surgeries was as follows: AH 0.74, VH 0.45, ELG 0.57, ELS 0.58, WP 0.49, FP 0.42, Splenectomy 0.41, THR 0.96, TKR 0.94.

Summary/Conclusions: The study shows that MSBOS for all nine elective surgeries is less than one which is much lower than the actual PRBC requisitions being sent for these elective surgeries. Keeping in view the large volume of surgeries in the institute, implementation of MSBOS would have huge benefits in terms of efficient use of blood and blood bank resources. This study provides evidence for formulation of MSBOS based blood utilization policy at institute level.

P-046

APPROPRIATENESS OF REQUESTS FOR BLOOD COMPONENTS: MONITORING OF THE YEAR 2016

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Background: One of the main point of the quality management system of an Immunohematology Service is the reduction of inappropriate use of the blood in order to improve the transfusions of red cells, platelets and fresh frozen plasma (FFP) and to reduce the associated transfusion reactions.
Aims: The aim of our study was to evaluate the effect of prospective monitoring on appropriateness of transfusions of red cells, platelets and FFP by the analysis of the blood components requests.
Methods: A retrospective analysis of the he transfusion requests of red cells, platelets and FFP during one year between January 2016 and June 2016 was executed. The requests were subdivided by month, department and type of blood component (red blood cells, platelets, FFP). The correct and incorrect requests were analyzed as follows: inconsistent personal data, absence of a history of blood transfusion and/or blood parameters pre and post transfusions, signature of the doctors, type of request (urgent, not urgent), diagnosis, requesting of an amount of blood products over-or underestimated compared to the indication, blood units withdrawn after many hours of the requests, no withdrawal of the blood components required. Requests were then monitored by blood bank laboratory staff for conformation with European transfusion guidelines; non-conforming requests were discussed with the *Committee good use of blood* of the hospital. A corrective action was then applied consisting to send a written communication to hospital departments in order to achieve greater rigor in the compilation of requests. A comparison by the previous transfusion requests and the subsequent one, made from July 2016 to December 2016, was then performed.
Results: A total of 1,498 transfusion requests of red cells, platelets and FFP occurred from January to June 2016, was analyzed. A total of 105 (7%) of non-conforming requests (NCR) were found. The majority of NCR were related to the lack of laboratory data (PT, aPTT, INR for FFP; Hb for RBC, PLT count and TEG for the platelets) and to the lack of the indication of the departments (especially surgical). After intervention, whereas, the rates of inappropriate transfusion requests fell significantly in the period from July to December 2016, and it was achieved a reduction of 15% of the NCR.
Summary/Conclusions: The data of our study shows that the prospective monitoring of request forms can reduce rates of inappropriate transfusions. Furthermore, the consistent application and monitoring of the guidelines provided by *Committee good use of blood* of the hospital allowed to an overall improvement of the hematological therapies.

P-047

IMPORTANCE OF CROSS MATCH TRANSFUSION RATIO IN BLOOD TRANSFUSION SERVICES IN A TERTIARY CARE HOSPITAL

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Background: A sufficient number of ABO and Rh compatible blood units should be available to meet routine hospital needs, allow for unanticipated increases in utilization due to emergency situations and minimizing component outdating.
The crossmatch/ to transfusion (C:/T) ratio is an important measure that is used to assess the quantity of the ordered crossmatched red blood cell units by the transfusion laboratory service. The C:T ratio is calculated by dividing the number of crossmatched red blood cell (RBC) units by the number of RBC units transfused. When cross match to transfusion (C:T) ratios are monitored a C:T ratio of >2 may indicate excessive ordering of crossmatched blood.
Excessive cross matching in addition to being wasteful of resources has adverse consequences on management of blood inventory and blood quality.
Aims: The main aim of this study was to analyse the pattern of blood cross matching and transfusion requests requirements with the aim of creating updated local policies that minimize resource wastage.
Methods: 60 months of retrospective data were collected which included RBC crossmatch requirements requests and all RBC units transfused.
Exclusion criteria were emergency transfusion crossmatch and issuance.
Results: A total of 30,814 units of blood were crossmatched and 18,473 units were used.
The overall C:T ratio was 1.71 corresponding to 58.39% of red cell usage.
Summary/Conclusions: The primary and secondary outcomes of the study was compliance with the international guidelines.
Furthermore, we also found that one approach to reducing excessive C:T ratios can be identifying procedures that do not typically require blood and use this information to develop guidelines. Maximum surgical blood orders for common elective procedures can also be restricted and adjusted to the local transfusion utilization needs. Once a surgical blood ordering schedule has been established the transfusion services can routinely crossmatch the predicted number of units of each patient undergoing the designated procedures.
Moreover, routine orders may need to be modified for patients with anaemia, bleeding disorders or other acute conditions in which rapid and increased availability of blood components is anticipated. Therefore the transfusion service staff should be prepared to provide additional blood components if the need arises.

P-048

BENCHMARKING REGIONAL HOSPITALS ABO BLOOD TYPE TRANSFUSION PRACTICE

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Background: Quality in transfusion practice applies to the hospital blood banks and it plays a key role in ensuring that the correct blood component is supplied for the correct patient.
A full crossmatch test is performed and it allows to exclude ABO incompatibility. In extremely urgent critical situations where blood is needed non-crossmatched group O blood may be available for immediate use.
However, in some peripheral and distant hospital blood banks, there may be a risk of over-utilization of some blood types over others.
The inadequate practice may therefore increase wastage in the supplier blood establishment.
Aims: To understand the red cells ABO type transfusion practice in hospitals blood banks of the region accordingly to the regional ABO type collections.
Methods: All data concerning blood collections and blood supplied to eleven hospitals blood banks of the region in the years of 2006 and 2015 were extracted from the Blood and Transplant Centre of Coimbra's in-house Blood Establishment Computer System.
These data were analysed by blood type concerning collection and supplied to the hospital blood banks.
In order to sensitize the good use of blood, the Portuguese Blood and Transplant Institute maintain online a web based tool where Blood Establishments and Hospital Blood Banks can access in real time the following data among others:

- National blood stock inventory (Blood Establishments and Hospital Blood Banks)
- Red blood cells (RC) units use in patients (Hospital Blood Banks).

Since 2006, technical audits to all hospital blood banks of our region are performed annually by the Blood Establishment team recommending Good clinical practice and the identical red cells ABO type transfusion practice is a major concern.
Results: Benchmarking hospitals red cells ABO type transfusion practice of our region, the data of the years 2006 and 2015 shows improvement trend for the O negative and A negative blood type use, with collection and use values being approximated, $P = 0.077$.
In overall of ABO blood types, it shows improvement trend as well, collection and use values being approximated, $P = 0.061$.
Summary/Conclusions: These findings show that in our region, the Blood Establishment team performing technical audits to all hospital blood banks contributes to a good transfusion practice, narrowing the gap between ABO type collections and blood use.
It suggests however the continuous need of monitoring the blood banks with longer distance to be supplied.
The knowledge of the National Blood Stock level seems to contribute to avoid stocking blood because it decrease the sense of insecurity due to shortage of blood.

P-049

EVALUATION OF RED CELL CONCENTRATES AND DONORS FOUND TO BE DIRECT ANTIGLOBULIN TEST POSITIVE

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Background: The screening of blood donations for direct antiglobulin test (DAT) is not mandatory, but when detected, the laboratory investigation is time-consuming

and may lead to discard of blood units. Management of positive DAT donors can also be problematic as there is no consensus regarding their acceptance.
Aims: To evaluate positive DAT Red Cell Concentrates (RCC) and respective donors.
Methods: From July 2016 to February 2017 an evaluation of positive DAT RCC reports was carried out. It involved RCC returned from Hospitals that were identified as having positive DAT and RCC blocked by the Informatic System (ASIS) because of positive DAT in previous donations. All RCC were hold for DAT performance, discarded if confirmed positive and released into stock for re-issue if negative. Donor files were reviewed. In case of negative results associated with the absence of positive DAT in donor records, Hospitals were identified and their results considered false positive. RCC coming from other Centers were no further investigated. Information of RCC and respective donors were assessed through ASIS and data, like gender, age, number of donations, frequency and type of positivity was collected.
Results: There were 77 reports, consisting of 52 returns and 25 retentions. After DAT performance, 55 RCC were discarded and 22 were released into inventory. Fifteen returns were RCC from other Centers, the outcome being 14 discards and 1 release into stock. False positive results were 14 and 1 Hospital accounted for 8 of them. The remaining 48 RCC were the object of this study and resulted in 41 discards and 7 releases. The corresponding donors were 38, 23 females and 15 males, mean age 44 ranging from to 23 to 67 years old. None was a first time donor. In this period, 6 donors donated blood twice and 2 donors 3 times. With the exception of 2 female donors sometimes deferred for low hemoglobin levels, donors were regularly accepted during the medical interview. The detection of positive DAT for the first time occurred in 14 donors, while the remaining 24 showed a positive DAT profile ranging from 5 months to 10 years of evolution with frequent discards. DAT study was enlarged in 20 donors, 15 having reactivity for IgG and 5 for C3d. Nine donors had a positive indirect antiglobulin test. Clinical significant antibodies were identified in 2 of them.
Summary/Conclusions: The rate of positive DAT confirmation was 71%. There were some false positive results and 1 Hospital was referred for training/counselling provision. After the revision of the 38 donor profiles, it was found that 14 had an incidental positive DAT detected in Hospitals most likely during compatibility tests. They can probably continue to donate blood as long as this is an isolated finding and pass the medical examination. The other 24 donors had a background of positive DAT associated with discards and although being in good health, they should be deferred from donation. The number of positive TAD allowed for a donor to continue to donate blood is a matter of debate.

P-050

REASONS FOR DISCARDING OF BLOOD AS QUALITY INDICATORS

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Background: The implementation of quality system and continuous evaluation of all activities of the National Institute of Transfusion Medicine (NITM) can help to achieve the maximum quantity and quality of safe blood. Optimizing blood collection and processing would reduce the rate of discard and improve the efficiency of the NITM.
Aims: The objective of this study is to compare the rates and reasons of discard of blood and blood component at the NITM during the period of 12 years (2005–2016) in order to evaluate the quality of the existing practices and to suggest additional appropriate interventions.
Methods: This is a retrospective and comparative study in which the data on the number of collected and discarded blood units, as well as the reasons for discard were obtained from the NITM-Information System and were analyzed. All the data were summarized in 4 subsequent 3 year periods: Period 1 (2005–2007), Period 2 (2008–2010), Period 3 (2011–2013) and Period 4 (2014–2016).
Results: The total number of collected/discarded blood units in the period 1, 2, 3 and 4 was: 56,700/4,719 (8.3%), 67,000/5,245 (7.8%), 91,600/7620 (8.3%) and 92,650/9,169 (9.8%) respectively. The major cause of discard was expiration of blood components (RBC) which increased from 4.4% (2,510) in Period 1 to 5.6% (5,209) in Period 4. The total number of discarded units due to other reasons (TTI reactive samples, positive RBC antibody screening, insufficient volume of the unit, inadequate blood samples for testing because of lipemia, hemolysis and mislabeling) did not change significantly over the analyzed period 1–4 being 3.8% (2,209), 3.6% (2,373), 3.7% (3,465) and 4.2% (3,960) respectively. The most frequent reasons for discarding of blood were the following: insufficient volume of the whole blood unit (16.4%), inadequate blood testing samples (6.1%) and lipemic samples (34.3%). The wasted blood because of insufficient volume increase from 4.3% (96) in Period 1, 11.6% (277) in Period 2, 18.6% (645) in Period 3 to 31.2% (1234) in Period 4. Non-conformities of blood testing samples decreased from 9.2% (204) in Period 1, 7.6% (182) in Period 2, 4.9% (171) in Period 3 to 3.0% (119) in Period 4. The frequency of lipemic blood samples did not change over the time but it is still the most frequent single cause of wastage of blood being: 24.3% (537), 34.8% (828), 47.1% (1635) and 31.2% (1235) in the Period 1, 2, 3 and 4 respectively.
Summary/Conclusions: The analysis of the reasons for blood discarding is useful for evaluating service demands, blood collection and production, adequacy of personnel, inventory control, and process stability. What we learned is that additional efforts should be made toward planning of the time and quantity of the collected blood according to the needs, efficacy of the inventory management, as well as continuous donor and NITM-staff education.

P-051

RATIONAL USE OF PLATELETS IN DENGUE MALARIA EPIDEMICS

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Background: Critical shortage of platelets during dengue epidemic leads to preferential transfusion of platelets.
Aims: To overcome platelet shortage during Dengue/Malaria epidemic in India.
Methods: 540 patients with high grade fever tested positive for Dengue / Malaria were admitted and screened for Malaria, Dengue (Ns IgM and IgG), CBC for Atypical lymphocytes(ATL), Platelet Count and Mean Platelet Volume(MPV).
Results: 336 patients had low platelet counts, 276 showed Atypical Lymphocytes (ALT), 182 had high mean platelet volume (MPV) and 94 had Normal MPV.
CBC samples were tested every ten hours, revealed that the patients with high MPV, increased platelet counts over the period of next 24 to 36 h, also their smear showed reduction in ATL.
However, patients with thrombocytopenia associated with ATL and normal platelet volume, worsened and started developing petechiae, these required immediate platelet transfusion. Thrombocytopenia patients with normal platelet volume associated ATL were also Dengue NS 1 and IgM positive, indicating active recent Dengue infection.
Summary/Conclusions: All Dengue, Malaria Cases with low platelets need not be transfused. Patients with Positive NS1, IgG, IgM, ATL having thrombocytopenia platelets not responding to production of large platelets, should be given immediate platelet transfusion. Such patients constitute to only about 27.9% as seen in this study, thereby releasing the remaining 70% platelets, as rational use requirement for the needy.

P-052

DISTRIBUTION OF ABO AND RH BLOOD GROUPS AMONG MAJOR ETHNIC GROUPS IN MYANMAR

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Background: It was known that the distribution of blood groups varies among ethnic groups throughout the world. Knowing the blood group distribution among each ethnic group in the country is fundamental for well-organized blood program.
Aims: The aim of the study was to describe the distribution of blood groups among ten major ethnic groups in Myanmar.
Methods: The people who came to mass donation during January to February 2017, and whose ethnicity is clear for two generations, were approached to participated in the study. After getting the consent, specimens collected in the mass donation were sent to National Blood Center for further examination for blood grouping including ABO, Rh(D, C, E, c, e), and MiIII (Gp. Mur), using the antigen antibody agglutination test of tube method.
Results: Total 863 ethnic people from Burma (100), Chin (100), Dabu (39), Kachin (110), Kayar (100), Kayin (70), Mon (53), PaOh (69), Rakhine (100), and Shan (122)

communities were participated in the study. Among Burmese to which more than 60% of the Myanmar population belongs, type B is most common as 37% of them, followed by type O (34%). Among Chin, and Mon, type A is dominant (38% and 49% respectively), followed by type O (32%. and 23% respectively). Among Kachin and Kayin, type O is dominant (44% and 37% respectively), followed by type A (34% and 26% respectively). Type O is also dominant among Kayer and Shan (65% and 40% respectively), but followed by type B (17% and 27% respectively). Among Rakhine, frequency of type A, B, and O are almost similar as 29%, 33%, and 32% respectively. Very few Rh D negative cases were observed in Rakhine (2 cases) and Chin (1 case) in the study. MiIII positive were only found in Kachin (3.6%), Mon (5.7%), and Shan (1.9%).

Summary/Conclusions: Significant variation was found in ten ethic groups in Myanmar. Rh D negatives and MiIII positives were only found in few ethnic groups in the study. This vital information may help the planning for strengthening the blood program, although we need further studies including frequency of other irregular antibody among those ethnic groups.

P-053

MOVING TO CENTRALIZED PLATELET PRODUCTION: EFFECTS ON WASTAGE AND UTILIZATION

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Background: Reducing blood product wastage is a major focus for all transfusion laboratories and blood bank. Platelet stock is at high risks of wastage due to the short shelf life of platelet and the variable clinical demand in hospital. In 2016 our Blood Transfusion Service has externalised the production of whole blood derived platelets and apheresis platelets in an other facility. This facility produces whole blood derived platelets and apheresis platelets also for other Blood Transfusion Services. The platelets had to be ordered from the blood supplier and delivered via a dedicated courier.

Aims: We studied indexes of efficiency of platelet logistics and clinical use for ascertain the need to enhance platelet inventory management operations.

Methods: Our blood center serves a community of three hospital including haematology departments, cardiovascular surgery departments, a solid organ transplant program, a trauma center and outpatient transfusion clinics. In 2015 our blood center produced 5,063 whole blood derived platelets and 1,230 apheresis platelets. In June 2016 the platelet production was outsourced. We assessed wastage rates, transfusion rates of Rh positive platelets to Rh negative patients, transfusion rates of AB0 mismatched platelets and days of shelf life of transfused platelets. We reviewed the period 15 June 2015–01 March 2016 (in house platelet production) and 15 June 2016–01 March 2017 (outsourced platelet production). In the second period we adopted a stringent policy for ordering platelet including an inventory check twice a day. A dedicated educational program was offered to all technician involved in platelets inventory management and issuing. A set of predefined data extracted from the database (inventory, programmed transfusions, crossmatched or washed platelets required, blood donor drives scheduled) was implemented and easily derivable from our management software in real time.

Results: In the first period were delivered 4,311 platelets with a wastage rate of 9.9%. In the second period we assigned 4,618 platelets and the wastage rate, after a meaningful decline, has increased to a final 10.3%. The rate of AB0 mismatched platelet issued was unchanged (3.4%). The rate of Rh mismatches has declined in the last period from 6 to 2%. Platelets issued in 2015/2016 in the first three day of storage were 58%. Platelets delivered in the first three days of storage increased to 68.2% in 2016/2017. The rate of platelets issued at the fifth day of storage time was 4% in 2015/2016 and decreases to 0.2% in 2016/2017.

Summary/Conclusions: Centralized production of platelets did not reduce platelets wastage. To some extent a certain rate of wastage is inevitable given the perishability of platelets and the often unpredictable needs for surgery, trauma, transplants and outpatients. An initial improvement of wastage rate was not confirmed in a longer period. Besides a reinforcement of the current procedures, maybe could be useful to adopt a bi-directional transfer between end users and production facility. The measures adopted were useful in improving other important areas of clinical practice: age of transfused products and AB0 or Rh mismatches between platelets and patient.

P-054

Abstract has been withdrawn.

P-055

METHOD OF DONATION AS FACTOR INFLUENCING INVENTORY LEVELS OF RED BLOOD CELL CONCENTRATE IN POLISH BLOOD ESTABLISHMENTS

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Background: Blood and blood components are essential therapeutic agents the adequate supply of which depends almost entirely on donor recruitment and efficient management of inventories in blood transfusion centers.

Aims: To evaluate the availability of whole blood donors as the primary determinant of red blood cell concentrate (RBC) inventory.

Methods: Institute of Hematology and Transfusion Medicine (IHTM) – as the supervising competent authority – performed the analysis of 2013–2015 data reported by the 23 Polish blood transfusion centers.

Results: Since 2010, there has been no marked increase in the number of Polish blood donors (605–630 thousand); a decreasing tendency observed between 2013–2014 (as compared to 2012). A decrease was also reported for first time donors (35% in 2010 – 25% in 2015). This may seem positive as the . . .donor pool" remains constant but the observed tendency is the cause for anxiety as it implies that new donors are not willing to join the "donor-pool". The number of donations has slightly increased during the last several years (from 1 180 thousand in 2010 to 1,275 thousand in 2015) with a reported modest decrease in blood component supply (mainly RBC) probably due to growing demand for blood component therapy.

The main component currently collected in Poland is whole blood, the common source of RBCs for therapeutic use. Since 2011 the number of whole blood donations slightly exceeds 30 per 1,000 inhabitants. The indicator correlates with periodic shortages in RBCs.

Blood transfusion centers in Poland are currently involved in activities related to increasing the volume of plasma collected by apheresis. The volume of apheresis plasma has been growing from year to year (from 19,855 units in 2011 to 45,126 in 2015). On the other hand the volume of plasma issued for clinical use decreases (390,022 units in 2011, 334,454 in 2015) which is a good sign given the limited indications for its clinical use.

Plasma donations are more popular with blood donors as the procedure can be performed even twice a month (whole blood donations require 8 week intervals) so they can sooner reach the target of donation-volume that qualifies them for awards and distinctions.

Reports on 2013–2015 donations presented how the increase in the number of apheresis collections affected the number of whole blood collections with negative effect on the coverage of clinical needs. This was particularly true in the summertime when appeals for whole blood donations became frequent; regular apheresis donors however rarely responded to such appeals. This explains the efforts undertaken to increase both the number of donors and whole blood donations.

Summary/Conclusions: Too many donors resigning from whole blood donation may pose a serious challenge to meeting the growing clinical needs in the nearest future. The critical issue therefore is effective management of blood donors, whole blood collections, convincing donors to consent to certain blood collection procedures (whole blood instead of apheresis plasma).

P-056

TRACEABILITY OF BLOOD PRODUCTS AT KORHOGO REGIONAL HOSPITAL IN COTE D'IVOIRE

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Background: Blood transfusion is a substitution therapy that saves many lives. This has not always been so. The transfusion has been and is the cause of many accidents and incidents.

The traceability of blood transfusion activities has highlighted the positive and negative aspects and today improve the safety of transfusions.
In order to improve blood transfusion safety, we felt it was necessary to evaluate the traceability in the hospitals of Côte d'Ivoire a few years after setting up hemovigilance committees.
Aims: The aim of this work is to establish the link between the blood bag, the blood donor and patients who receive blood products.
Methods: Retrospective study, at the Regional Hospital Center of Korhogo in Côte d'Ivoire, from 01 September 2013 to 30 June 2015.
It covered all blood products delivered by the Korhogo transfusion center. The distribution data for blood products from the transfusion center to Korhogo hospital were extracted from the NBTC medical software (PROGESA). The data of blood products delivery by the Hospital's Blood Bank is available on a paper register. Data on transfusions in the care units are available on transfusion surveillance sheets. The data on the distribution and delivery of the blood product and the data on the transfusions are entered in the same data base designed for the circumstance. The cross-references of these three data sources, as well as the counts, were made using queries made with this Access data base.
Results: At the quantitative level: The transfusion center distributed 7,733 red blood cells, the hospital blood bank traced the issuance of 6,210 blood products and care units have transfused and traced 3,531 (46%).
At the qualitative level: 1,269 out of 7,733 blood products are found and identified unequivocally (blood bag number, product and blood group ABO RhD) or 16.4%. In total, the link GIFT DONOR RECEIVER (unequivocal identification of the product and receiver) is established in 720 cases, ie 9.3%.
Summary/Conclusions: The link GIFT DONOR RECEIVER established will allow to be able to carry out hemovigilance investigations and to improve the safety of blood transfusions.

P-057

Abstract has been withdrawn.

P-058

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P-059

EFFECTIVE AND SUCCESSFUL STRATEGIES IN PROVIDING ADEQUATE AND SAFE BLOOD SUPPLY IN KHOUZESTAN BLOOD TRANSFUSION SERVICE (6 YEARS EXPERIENCE)

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Background: Nowadays, by advances in medical sciences, the blood transfusion organizations play vital role in public health. Due to increase the demand of blood and blood components, providing safe and adequate blood supply need great efforts and attention. Until 2006 our province provide some of its blood supply by family replacement donation. Due to high number of blood depended patients in our province, providing totally voluntary non remuneration was difficult mission for our organization.
Aims: Evaluating and monitoring the trend of blood donation rate and type and Hepatitis marker during 6 years helps us to effectiveness and success in providing safe and adequate blood safety.
Methods: Our study was retrospective cross sectional study and included all blood donors admitted to Khouzestan blood transfusion service during 2008 to March 2016 by nonrandom simple sampling. For all blood donors the viral markers checked by using Enzyme Immuno Assay methods and repeatedly reactive samples were checked by confirmatory tests. The data analyzed by SPSS software.
Results: We found that 1,205,305 people admitted for blood donations and just 66% and 76% in first and last year of our study respectively. The donation rate was 25 per 1,000 population. The regular donation significantly increase from 25.9% to 66.9% in start and end of our study respectively and at the same time the Hepatitis marker showed the significant increase from 0.67% to 0.16%.
Summary/Conclusions: According our study, our mission in providing safe and adequate blood supply was difficult but effective and successful. Implementation and following our strategies and plans highly recommended for countries with difficulties in blood supply.

Quality management

P-060

EVALUATION OF AN IRRADIATION INDICATOR WITH BARCODE READ-OUT BY THE BLOOD SERVICE OF THE BELGIAN RED CROSS-FLANDERS

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Background: Irradiation of red blood cell concentrates is necessary to destroy the DNA in residual white blood cells, particularly lymphocytes that could cause transfusion-associated graft versus host disease in immunocompromised patients. Irradiation indicators are a valuable tool to verify the irradiation of blood products. A standard irradiation indicator (25 gray (Gy)) affixed to red blood cell products is currently used to provide positive, visual verification of irradiation. This visual reading is then used to enter the irradiated status of the blood bag in the blood bank information system (BIS). In collaboration with the Belgian Red Cross-Flanders, On Point developed a new type of label – modified RAD-CONTROL – that also signals exposure to the required dose of irradiation by the appearance of a specific barcode.
Aims: The Blood Service of Belgian Red Cross-Flanders performed a study to evaluate a custom-made irradiation indicator that allows read-out of the irradiation status by barcode scanning. More specifically, the readability of the barcode and the adhesive performance of the label after various storage conditions were tested.
Methods: The modified RAD-CONTROL label was used to indicate exposure to gamma or X-ray irradiation of at least 25 Gy. After a blood product and its attached indicator are irradiated, the word "IRRADIATED" appears in one window of the indicator and a barcode meaning "IRRADIATION OK" becomes visible in a second window. On scanning of this barcode, combined with the scanning of the unit number of the blood bag, the latter acquires the status "Irradiated" in BIS. Sixteen dummy bags with a modified RAD-CONTROL label attached on the bag label, next to the unit number, were sent to 2 different radiotherapy services for irradiation with a linear accelerator (X-ray). Four other bags plus indicator were irradiated at our production site of Campus Mechelen using a gamma irradiation apparatus (MDS Nordion Gammacell 3000 Elan). The appearing of the barcode and of the word "IRRADIATED" after irradiation was evaluated, as well as the readability of the barcode using a scanner immediately after the irradiation and after storage at 4°C for 28 days. We also evaluated the adhesive force of the label immediately after applying it and at the end of storage.
Results: In total, 20 dummy bags were irradiated using the modified RAD-CONTROL indicator. All dummy bags were correctly irradiated. For every bag, the barcode appeared in the upper red section and the word "IRRADIATED" in the lower red section. Eight of the 20 irradiated dummy bags were stored during 28 days at a temperature between 2 and 6°C. The readability of both barcode and textual "IRRADIATED" indications were still good at the end of storage. The adhesive power of the label was good at the start, but showed some minor deficits after 28 days of storage where overlapping the unit number label. The barcode could be scanned correctly, but the alignment of the "IRRADIATED" barcode with the unit number barcode caused some mix-up of the scan-read information in our blood bank information system.
Summary/Conclusions: The barcode read-out of the irradiation indicator proves feasible. This can enhance the reliability of the registration of the irradiation, and as such contribute to the safety of our processes. Further improvements are needed of the adhesive force at the end of storage and of the position of the "IRRADIATED" barcode in relation to the unit number barcode.

P-061

MEDICAL DEVICE PROBLEMS REPORTED IN THREE DIFFERENT REPORTING AND LEARNING SYSTEMS

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Background: Extensive use of medical devices and laboratory information systems are necessary in blood banks and transfusion services. In Norway we have 200.000 blood donations annually and 260.000 blood components are transfused (red cell concentrates, plasma and platelet concentrates). Norway has a national haemovigilance system as required in the EU Blood Directives. In the haemovigilance system reports come mainly from blood bank staff. We also have a RLS specific for medical devices as required in EU directives. Reports in this system are submitted both by hospital staff and by manufacturers. In addition we have a universal reporting and learning system (RLS) for serious adverse events, including near misses, in specialized health care institutions. Reports in this system are submitted within 24 h after detection and all hospital staff are allowed, and required, to submit such reports.
Aims: The objective of this study was to identify what was reported from the transfusion services about medical devices and laboratory information systems in the three different RLS.
Methods: We used the databases of the three RLS to identify reports of medical device problems and laboratory information system problems related to blood donation and blood transfusion, received between January 1st and December 1st 2016.
Results: In the haemovigilance system we had 537 reports, all related to blood donation or transfusion, but only 11 were classified as medical device problems or laboratory information system problems. In the medical device RLS there were 195 reports. Only three reports were about problems related to blood donation or transfusion. One was submitted by the manufacturer. The two other reports were about the same incidence, reported both by the blood bank and by the manufacturer. This problem was in addition reported to the haemovigilance system. In the universal RLS we had approximately 10.000 reports. 597 were classified as medical device related and 103 were related to blood donation or transfusion, but only three reports were related to both. In addition two reports were related to both blood transfusion and to "products, technology and infrastructure". One of these was also reported in the haemovigilance system. Hence, the 19 reports on medical device problems and laboratory information system problems identified 16 different incidents. The reports covered a variety of problems. Seven reports were about the laboratory information systems not performing as expected, such as not automatically ordering the required testing for infectious agents and not alerting staff when incompatible blood was issued. Three reports were blood bags leaking or having additive solutions in the wrong bag. Two reports were related to the needle falling off when being withdrawn from the vein or injuring the vein because of a production error. Two reports were about equipment not being available when needed in an emergency situation. One report was about blood typing reagents having the wrong label. One report was about the pneumatic tube system used to transport blood components from the blood bank to the wards malfunctioning.
Summary/Conclusions: Three different RLS give different results and complement each other, as they give information about different types of problems. In our view reporting to a national RLS is useful because other users of the same devices can be informed about the problem. Reporting problems to a national RLS may strengthen the customer, especially if the same problem is reported by several blood banks.

P-062

BUILDING A PROACTIVE INFORMATION MECHANISM TO OVERCOME TRANSFUSION CHALLENGES IN PATIENTS UNDERGOING ABO-INCOMPATIBLE HEMATOPOIETIC PROGENITOR CELL TRANSPLANTATION

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Background: Transfusion for patients undergoing ABO blood type incompatible (ABOi) hematopoietic progenitor cell transplantation (HCT) represents a distinct set of challenges for transfusion services. The potential problems involve selection of different blood group components with regard to mismatching direction and engraftment status.
Aims: The aim is to build a proactive information mechanism to overcome transfusion challenges for patients undergoing ABOi allo-HCT at our hospital.
Methods: In January 2011, we began with an activity of Healthcare Failure Mode and Effect Analysis to identify the potential transfusion risks for ABOi HCT recipients and designed preventive programs integrated into transfusion support information system (TSIS) to target the major risks. There were two stages to the program design. At the first stage, we compiled lists of allo-HCT donor/recipient pairs and integrate the transfusion algorithm for ABOi HCT into the TSIS, with alert functions regarding patient's transplantation status and the recommended blood groups for different components (completed in September 2011). The second stage was to build a check engine into the TSIS to ensure the blood group of components be issued according to the recommended guideline (completed in September 2014).
Results: From Jan 2010 to Dec 2016, a total of 691 patients underwent allo-HCT in our hospital. Of them, 50% belonged to ABOi HCT (incompatibility: bidirectional 7%, major 20%, minor 23%). The percentage of ABOi is higher in unrelated donor/recipient pairs (60%).We monitored the numbers of transfusion error and near miss involving transfusions in cases of ABOi HCT during different stages according the programs implemented (three stages: January 2010 – August 2011, September 2011 – August 2014, and September 2014 – December 2016). During the first stage (20 months), 1 case of near-miss and 3 cases of error were reported. Three cases of near-misses were detected after the implementation of the alert-system (36 months). Only 1 case was noted after the implantation of the additional check function (28 months), and the cause for the near-miss was failure to enlisting the patient in the ABOi database of the TSIS.
Summary/Conclusions: We have established an effective transfusion issuing engine system to ensure the transfusion safety of patients receiving ABOi HCT. The safety engine has also been modified for recipients undergoing ABOi organ transplantation since 2016.

P-063

EVALUATION OF SOME OF THE QUALITY INDICATORS IN THE NATIONAL INSTITUTE FOR TRANSFUSION MEDICINE IN THE REPUBLIC OF MACEDONIA DURING A SEVEN YEARS PERIOD (2010–2016)

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Background: A 7 years retrospective analysis (2010–2016) of the quality indicators (QI) of the preparative process, stock and distribution of blood components in the Institute of Transfusion Medicine in Skopje, Republic of Macedonia, established with the introduction of the Quality Assurance and Quality Control system since 2007, was done. The process was started with the EU Project Safe Blood with the help of French government in the legislative harmonisation with EU Blood Directives, finished in 2010, and with IPA Project "Strengthening of the Blood Safety System" (2012–2014) when an unique IT, component processing and stock equipment for the all Regional BT centers in the country was achieved and the whole personnel was trained in a Quality Management Course.
Aims: To evaluate some of the quality indicators in the transfusion medicine after the reorganization of the Institute of Transfusion Medicine in Macedonia in an unique institution and the introduction of QA/QC since 2010.
Methods: The QA/QC monthly and yearly reports and records for out-dated blood components, non-separated whole blood, rejected components during processing, leuco-depleted blood components and non-conformities were evaluated in the study.
Results: Outdated RBC-SAG ranged from 2.19% in 2010 to 5.00% in 2016, outdated PLT single donor concentrates 17.13% in 2010 to 15.57% in 2016; unseparated whole blood 2.36% in 2010 to 3.84% in 2016. Leuco-depleted components- Filtrated RBC concentrates were constant the whole investigated period: 4.84% in 2011, to 4.39% in 2016, filtrated PLT – 0.81% in 2012, to 1.12% in 2016, BC PLT production increased from 56 in 2010, to 122 in 2013. The reports of adverse reactions were the whole period the same from 1 to 2 in a year, mostly mild, allergic, to FFP, cryo and RBC-SAG, only one haemolytic, with detected Fy atb.
Summary/Conclusions: The analysis of the quality indicators in our BT service presented that the % of processed whole blood into components (RBC, PLT, FFP, CRYO). from approx. 97.7%) during the first three years of the investigated period falled on

95.99% in 2015, resp. 95.61% in 2016. The outdating of RBC-SAG components is constantly increasing from 2.19% in 2010 to 5.00% in 2016, and outdating of PLT concentrates is decreasing, from 17.13% in 2010 to 15.57% in 2016, due to careful daily planning of the production according to the clinical requests and their fulfilling, indicating good management of PLT usage, The percentage of the rejected plasma components during processing decreased from 1.90 in 2010 to 0.90% in 2016. During the investigated period The reports for non-conformities ranged from 19 in 2010 to 36 in 2013, mean 25.5 reports per year, presenting 0.08% in 2010 and 0.17% in 2013, mean apx. 0.11% reported non-conformities of the whole collected blood in Skopje in that period. Most of them were coagula in RBC-SAG concentrates, leakage from broken FFP blood bag and wrong labelled RBC-SAG and FFP. Most of the non-conformities were due to the improper blood mixing during blood collection with the corrective action undertaken- training of the staff in blood donation procedure. The low number of adverse reactions is most probably due to non reporting from the clinics, but anyhow, no serious adverse reaction was reported during the whole investigated period. The introduction of QMS and regular training of the BT staff presented visible results and is constantly needed and recommended.

P-064

QUALITY OF SERVICES AND PATIENTS SATISFACTION AT NATIONAL INSTITUTE OF TRANSFUSION MEDICINE

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Background: The measurement of the quality of services is an important part of quality system. The National Institute of Transfusion Medicine (NITM) beside the blood bank services, provide as well out-patient services, with wide variety of analyses in immunohematology, haemostasis, HLA and molecular biology. With 65 physicians and 150 other medical staff, it is important to follow the quality of services and patient satisfaction.
Aims: To evaluate the patients satisfaction by the out-patient departments within the NITM and to point- out weak points that should be improved.
Methods: By retrospective study were analyzed data from the standardized questionnaire, designed and distributed by the Ministry of health. The comparative method was used to evaluate patient satisfaction for the period of two years (2015–2016). The questionnaire has 16 questions, out of which 3 are for general respondents data: age, sex and nationality; rest 13 questions are divided in 4 categories: (1) availability of services, (2) waiting time (for physician examination, waiting time for nurses to take blood samples for laboratory analysis), (3) the staff attitude toward patients and (4) overall satisfaction (comfort and safety and the probability that services will be recommended to someone else). Total of 510 respondents, voluntary and anonymously, have answered the questionnaire. The satisfaction was measured with five point Licker scale (1- not at all satisfied, 5- very satisfied).
Results: At all out-patient departments at NITM were registered 154.239 medical examinations (2015) vs. 169.994 in 2016. More females (63.7%) than males (33.3%) have answered the questionnaire (year 2015), while in 2016 the structure is almost equal (50.2% males and 49.8%). Dominant are patients at ages 60 or older (31.5% in the year 2015 vs. 30.9% in the year 2016). The ethnical structure: Macedonians (71.8%), Albanians (14.7%), Turks (5%), Serbian (2.7%) and other (5.8%). Respondents were very satisfied with the short period of waiting before medical examination (mark 5: 71% in 2015 vs. 70.2% in 2016); short waiting time for laboratory analysis (mark 5: 68.1% in 2015 and 67% in 2016) and waiting time to take the final results (mark 5: 57.9% in 2015 vs. 67.7% in 2016). The physicians good communication was evaluated as excellent by 82.6% (2015) of respondents vs. 81.5% (2016). The physicians give understandable explanations (mark 5: 78.2% in 2015 vs. 77.7% in 2016), and clear therapy recommendations (mark 5: 78.2% vs. 78.7%). The access to out-patient services was evaluated as excellent (55% vs. 61.3%). The lowest satisfaction was for comfort and safety during waiting time (mark 5: 27.5% in 2015 vs. 49.7% in 2016) as well as for hygiene (mark 5: 26% in 2015 vs. 55.9% n 2016).
Summary/Conclusions: As overall results, respondents have shown high level of satisfaction from received services, except for the comfort and hygiene. Taking in consideration the decreased number of cleaning staff, old buildings were out-patient services are situated, as well as the restricted financial resources, it can be understandable why those are weak points. However, managers have to find a solution for those pointed weaknesses.

P-065

USING REMOTE WEB BASED TECHNOLOGY TO IMPROVE BLOOD DONATION AND TRANSFUSION PRACTICE IN TWO AFRICAN COUNTRIES

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Background: A project was designed to improve blood donation and transfusion practice using remote web based data collection managed in the United Kingdom. The approach deployed readily available online data collection software used routinely within the NHS Blood and Transplant (NHSBT) service to support Patient Blood Management (PBM) initiatives. This aspect was linked to active service improvement projects on the ground in two African countries. A project was implemented in the Ugandan Blood Transfusion Service (UBTS) and the Ugandan Red Cross (URC), with support from the Global Blood Fund (GBF). The second project is in progress at the University Teaching Hospital in Lusaka, Zambia (UTHZ) with on-the-ground support provided by the Tropical Health and Education Trust (THET).
Aims: The aim was to provide reports and data that could be used locally to improve donor recruitment in the UBTS/URC and blood transfusion practice at UTHZ. For UBTS/URC these web tools were used to support a pilot to improve donor recruitment rates, by providing donor ABO/Rh typing at blood donation sessions using anti-sera reagents. This project was called "What's Your Type?" (WYT). In Zambia, reports were used to support training sessions at UTHZ to improve blood transfusion practice across two key areas:

- Management of major haemorrhage.
- Improvements in blood transfusion documentation.

Methods: The projects were supported by the NHSBT International Development Programme (NHSBTIDP) by providing data and report feedback in a usable format derived from information submitted via a web based interface from the two sites involved. Support was provided on a pro-bono basis. Data governance issues were discussed and addressed. For the UBTS/URC project, a 2-day training programme was delivered to the Nakasero collection team in Kampala, Uganda to train staff on the blood group typing process and online data entry. For the UTHZ project, communication was maintained via videoconferencing. Data forms were sent via email to NHSBT and re-designed for online data submission. Data could be entered on a case by case basis with a save function to preserve information in the event of internet or power outages. Both sites were able to access their own data and updatable reports by using a secure "Associate Account" login.
Results: For the UBTS/URC data collections, 1,460 valid donor datasets from 50 WYT sessions were submitted. Of the 50 sessions, 41 were conducted alongside an actual donor session where blood could be donated. Of the 1,460 donors that were typed, 604 went on to donate blood (41% recruitment to donor conversion rate). For UTHZ, 4 online data collection forms have been successfully deployed. Technical utility has been demonstrated by the submission of 230 datasets related to product tracking. Data relating to these have been fed back and accessed by UTHZ who are able to generate updateable summary reports for immediate review.
Summary/Conclusions: Readily available IT solutions can be deployed successfully in remote locations and facilitated locally from the UK. It is possible to support valuable service improvement initiatives overseas in a collaborative way with potential direct benefits to the user.

P-066

THE IMPACT OF STRUCTURED INCIDENT ANALYSIS ON CONTINUOUS WORK IMPROVEMENT IN BLOOD ESTABLISHMENT.

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Background: 1. Blood Safety and quality regulations that applies to blood establishments and hospital blood banks requires blood facilities to report, record and analyze incidents and near miss events to determine the required corrective actions and to prevent it's recurrence. Proper incidents and near misses management has a major role in continuous improvement of quality management.
Aims: 1. This study shows the results of analyzing the different types of incidents and events reported in Dubai Blood Donation center (DBDC) after structuring a comprehensive reporting and recording system that allows the quality management to identify the weakness and plan for corrective and preventive actions and evaluate its effectiveness by tracking and trending.

Methods: During 2016; DBDC. has started a new Deviations, Non-Conformances and Incident Reporting Procedure. The incident reporting form requires quality personnel to review and investigate then complete the form that includes: Incident Categorization(Pre-analytic, Analytic, Post Analytic), Contributing Factors (most common factors are listed), Severity Level Classification (Level 1–4 with definition for each). Tracking and trending of incidents is done by each Unit individually and findings are reported biannually for management review for further analysis and evaluation.
Results: During 2016; 49,223 donations were collected, processed, screened,and stored in DBDC. The reported incidents were 207. 55.6% were pre analytical, 25.6% analytical and 18.3% are post analytical. Within the pre analytical 46% were under: erroneous in blood donor registry;while erroneous in processing blood & components was the highest (18.9%) under analytical category and 42% of post analytical were under data entry error. While analyzing severity level: Level 1 was the highest (87.8%) in Medical Care Unit where blood donors are interviewed, checked and donate followed by 27% in processing & shipping Unit. While analyzing the contributing factors: 29.4% due to SOPs not followed, 19.6% due to carelessness, 17.3% records inadequately reviewed, 3% understaffed and 0.4% training required. The reported incidents were analyzed according to the related health care staff category where 46% of the incidents were conducted by nurses whom are at donors services and mainly due to incomplete data in donor history questionnaire .Then 32% by medical laboratory technologist and 14% by phlebotomists. Few incidents which was found to be caused by defect in SOPs; were not repeated after changing the related SOP. Staff overload was also detected during our analysis that brought our attention to assess the productivity of our processes.
Summary/Conclusions: The new structured incident management system is very useful for identifying the weakness in the system and help us through root cause analysis to do the required corrective action and prevent recurrence. Deviations and nonconforming in routine work can be due to an individual not conforming to applicable policy and procedures or due to a defect in the policy and procedure itself.

P-067

A STUDY ON THE IMPLEMENTATION AND STABLE ESTABLISHMENT OF GOOD MANUFACTURING PRACTICES FOR HUMAN BLOOD AND BLOOD COMPONENTS IN KOREA

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Background: Standards for the manufacturing and quality control of blood products were recently introduced in Korea. There is a need for establishment and implementation of good manufacturing practices (GMP) for human blood and blood components on Korean blood products manufactures.
Aims: The purpose of this study was to establish an appropriate GMP standard for characteristics of blood component preparation. It also aimed to provide a draft of commentary and inspection checklist for blood component product manufactory, and ultimately to settle down the safety management system of blood component production.
Methods: We performed comparative analysis of related laws and foreign cases by literature searching. Through literature review, 135 questionnaires on blood product GMP including quality management, personnel, documentation, premises and equipment, qualification and validation, management of materials and reagents, and manufacturing process were included on the survey and distributed to manufacturers of blood components. We conducted on-site visit of blood product manufacturers which are located in Korea and other countries and listened the opinions of the field personnel concerning GMP. This study was initiated and supported by the Korean Ministry of Food and Drug Safety.
Results: Overall, understanding and preparation of the GMP was as proper as expected. It was necessary, however, to train the validation process and to support facilities and personnel. After analysis of related laws and regulations, and acceptance of an opinion by public hearing, we created a draft of commentary for glossary, premises and equipment, organization, documentation, qualification and validation, manufacturing, health and safety, management of materials and reagents, complaints and product recall, change control, look-back, internal audits, and training. Inspection checklist containing proper plans was also produced.
Summary/Conclusions: For stable settlement of GMP in blood component manufacturing, we believe that continuing education on blood product manufacturers, mutual understanding between the manufacturers and regulatory authorities, and the exchange of information with foreign institutions would be necessary.

P-068

Abstract has been withdrawn.

P-069

ANALYSIS OF QUALITY INDICATORS IN THE CENTER OF BLOOD AND TRANSPLANTATION OF COIMBRA

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Background: Quality indicators is defined as the specific performance measurements designed to monitor one or more processes during a defined time and are useful for evaluating service demands, production, adequacy of personnel, inventory control, and process stability.
The implementation of quality system and continuous evaluation of all activities of the Blood Centers can help to achieve the maximum quantity and quality of safe blood. Optimizing blood collection and processing would reduce the rate of discard and improve the efficiency of the Blood Center.
Aims: Evaluating the results of the quality indicators implemented in the Blood and Transplantation Centre of Coimbra (CBTC) during the year of 2016 in order to introduce appropriate intervention.
Methods: Periodic analysis of quality indicators related to the production process, storage and release of blood components in our center, established in the Quality Management System.
Quality indicators monitored over a one-month period:

- Production, storage, release and inventory management of Red Blood Cells (RBC)
- Production, storage, release and inventory management of platelet components (PC).

Results:

	2016	Goal	Tolerance	Critical value	Rate of realization	Annual Classification
Number of RBCs produced (derived from WB)	131,105	137,000	13,700	150,800	100%	Reached
Number of platelets produced from WB donations	12,767	13,000	1,300	14,301	100%	Reached
Number of RBCs validated (WB derived)	54,838	60,000	6,000	66,010	100%	Reached
Number of platelet validated - APHERESIS DERIVED	375	330	33	364	133%	Surpassed
Number of platelet validated - POOLS	9,444	9,200	920	10,125	100%	Reached
Number of red cells issued (derived from WB donation)	53,641	58,800	5,880	58,801	100%	Reached
Number of platelet units issued (derived from WB donation).	373	330	33	364	132%	Surpassed
Number of platelet units issued (derived from APHERESIS donations)	9,401	9,200	920	9,293	100%	Reached
Number of red cells expiring	3.8%	6.0%	0.7%	5.3%	135%	Surpassed
Number of platelets expiring (derived from WB donation)	0.28%	0.50%	0.10%	0.4%	135%	Surpassed
Number of platelets expiring (derived from APHERESIS donations)	0.53%	1.00%	0.2%	0.8%	135%	Surpassed
Blood Supply Management	98.8%	96%	2%	99%	123%	Surpassed
Rate of discarded red blood cells (RBCs) during processing	0.3%	0.30%	0.090%	0.200%	100%	Reached
Rate of discarded platelet-Pools during processing steps	0.7%	0.90%	0.200%	0.600%	100%	Reached

Summary/Conclusions: The periodic analysis of quality indicators are important contribution in assessing the performance of objectives and the adequacy of the process.
The information provided facilitates the proposed actions to be taken to correct perceived trends and bring them to the target in a cycle of continuous improvement.
The quality indicators have interest in both for taking decisions and remember that what is being measured is actually what you have and what needs improvement.

P-070

PLATELET CONCENTRATES INVENTORY MANAGEMENT IN BLOOD TRANSFUSION INSTITUTE OF VOJVODINA

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Background: Blood and blood components are valuable resource, sometimes deficient despite efforts to ensure the sustainable supply. Using blood and blood components before their expiration date is important not only in terms of the patient treatment, but also in terms of cost-efficiency. Maintain stocks of blood and blood components are often a major challenge for the transfusion center. Inventory management of platelet concentrates (PC) is the biggest challenge because of their short shelf-life and variable demand. PC stock has a natural tendency to overproduce, which leads to high expiration rates.
Aims: To determinate the optimal level of PC inventory at the Blood Transfusion Institute of Vojvodina (BTIV) in order to ensure the sustainable supply for users.
Methods: One year retrospective analysis of the daily requirement for a PC, the number of whole blood donated units, the number of producing PC and the number of discarded PC because of outdated.
Results: BTIV collected 27,556 whole blood units during the 2016 year. Out of them 21,747 units were produced single PC (78.91%). The PC was produced: 8,973 (41.79%) of blood group A, 6,480 of O (30.18%), 4,150 of B (19.33%) and 2144 of AB (9.98%). An average of 1626.33 PC was producing monthly. At the same time we made 327 apheresis PC. When the input of whole blood was reduced the number of apheresis PC was greater. Total PC requirements were 27530. Minimum PC requirements per month were 1225 and the maximum was 3188. Due to the expiration 233 PC were rejected. The average monthly deficit was 668 PC. Part of the PC deficit was met with PC from other blood transfusion institutions.
Summary/Conclusions: We analyzed our strengths, weaknesses, opportunities and threats in order identify the roots of the problem (external in the clinic and internal at the Institute). The key elements are: a lack of timely information what are the needs of the clinics, inadequate daily plan to collect blood and consequently insufficient production PCs. Continuous monitoring of blood stock, improvement of the collection blood and the blood production process, following the requirements of PC, reducing discarding of components etc, can be helpful to solve problems.

P-071

EXPANDING THE SCOPE OF REGULAR QUALITY CONTROL FOR BLOOD, BLOOD PRODUCTS AND PROCESSES TO ENSURE BLOOD SAFETY, ETHIOPIAN NATIONAL BLOOD BANK SERVICE EXPERIENCE

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Background: The World Health Organization recommends blood banks to adhere to good manufacturing Practices at all levels of the manufacturing process, from the collection of the source materials to the quality control of the final product, thus ensuring traceability from the donor to the recipient. Thus, the Quality assurance and safety department of the National Blood Bank Service (NBBS) performs monthly quality control tests and reports the results to the blood bank management.
Aims: Results from the monthly quality control tests and checks done by the quality department and reported to the blood bank management from 2014 to 2016 was reviewed to appreciate the key improvements achieved by the continuous monitoring of these activities as stated in the national standard.
Methods: The quality department performs quality control tests to Whole blood, concentrated red cell units,Platelet concentrates and cryoprecipitate preparations taking sample from the total collection. The tests were Visual inspection and volume measurement to all products with an additional cell count test performed for the platelet concentrates. Starting August 2014 it was decided to expand the scope of the quality control and additional regular data collection was started including cold chain maintenance trends for blood transport and storage equipment; Sterility of Platelet units; Overweight and underweight blood units before and after processing; discard rates due to different reasons; functionality checks and corrective maintenance done for the blood collection mixers critical materials for blood collection and distribution.
Results: The amount of tests and checks done and reported for quality review to the management has increased significantly after these changes as the parameters increased to 15 from only 4. The total blood and blood products tested annually for volume and visual inspection has increased from 240 units to 780 units out of a total collection of 44,029 and 48,430 in 2014 and 2016 respectively. The total follow up of cold chain maintenance measured by the proper registration of temperature every six hours per day on the total 33 cold chain equipment in the blood center has reached 97.62% in December 2016 from 89.6% in the same month in 2015. On the transport of blood from mobile collection sessions to the central blood bank proper records on the acceptability of transport status and temperature found were for 130 (98.48%) mobile session out of 132 for the month of December 2016 which was only 29 records (40%) out of the 79 sessions conducted in December 2015.
Summary/Conclusions: Improvements can be seen from the expansion of the data collected for the monthly quality control tests for products and process in the national blood bank. These expansions of the reports to include the different processes in the blood bank has assisted the top management to easily identify areas that needed intervention and act on them in a timely manner to improve the quality of blood and blood products and the overall service.

P-072

PARTICIPATING IN AN EXTERNAL QUALITY ASSESSMENT SCHEME; THE ETHIOPIAN NATIONAL BLOOD BANK EXPERIENCE

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Background: According to WHO, quality assured testing of donated blood entails participation in proficiency testing and use of SOPs. Ethiopia has been participating in the one world accuracy proficiency testing scheme based in Vancouver, Canada since 2011 and had SOPs for laboratory testing since 2010. Ninety proficiency testing samples were received from the one world accuracy through the Ethiopian Public Health Institute (EPHI) per year in 3 rounds usually in March, July and October.
Aims: We reviewed the EQAS performance of the Addis Ababa Centre from 2011 to 2016 with the aim of demonstrating the importance of collaboration in improving quality of testing in blood transfusion services in resource limited settings.
Methods: Proficiency testing samples received from the EPHI, were tested by the laboratory staff at the NBBS Centre in Addis Ababa for HIV I&II, HBsAg, HCV antibody and syphilis antibody as well as blood grouping and Rh typing as part of routine testing. Results of the testing were then entered into an online tool, the one world accuracy system (OASYS) and were accessed by the one world accuracy team in Canada. The analysis by the EQAS lab was received through the same online system by those NBBS staff with access.
Results:: A total of 540 tests in 108 batches of 5 tests per parameter, were received for the above in the 6 years. Of these 13 batches were not tested. Of the 527 tests that were performed, all tests for HIV 1&2 and HCV were 100% accurate. All tests for blood group serology were accurate except for the 4 samples that were hemolysed and categorized as inappropriate upon receipt. Syphilis had 3 inaccurate results, 2 false positives and one false negative (96.7% accuracy). HBsAg had only 4 negative samples tested as positive (88.9% accuracy). Overall 99.06% of the total tests done were accurate.
Summary/Conclusions: Through the collaboration with the EPHI, a research institute with a lot of experience and extensive collaboration in the field of laboratory testing, NBBS has been able to participate in EQAS at no direct cost. The quality of laboratory testing has greatly improved in the centre and the participation in this same scheme will be extended to all the 24 regional blood banks in the country. This approach can be used in other countries with similar health care systems and social economic setting.

P-073

REGULAR AND CONTROLLED QUALITY MONITORING: ACHIEVING THE RELIABILITY AND ACCURACY OF THE EQUIPMENT

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Background: Quality monitoring is an essential procedure to evaluate the performance and accuracy of equipment used to detect the haemoglobin level of the donors (either capillary or venous blood). When a new device was used in the National Blood Transfusion Center for pre-donation capillary haemoglobin measurement, it was noticed that the routine quality monitoring done by calibrated venous blood cell counter, readings showed deviation from the new device's readings.
Aims: To study the significance of this deviation noticed between the new device and the standard quality monitoring device in order that a corrective action is to be taken to ensure an accurate measurement of the donors' haemoglobin.
Methods: Five hundred and eight randomly selected blood donors were studied. These represent 100% of the available population. Single fingerstick blood samples were obtained to determine the donors' haemoglobin levels by the test device, while venous blood samples were drawn for measurement of the haemoglobin level by a calibrated, automated haematology analyser as the reference method. According to the Egyptian National Blood Transfusion Standards, donors with a haemoglobin concentration in the range of (12.0–18.0 g/dl) for females and (13.0–18.0 g/dl) for males were accepted for blood donation. The system of the test device is based on photometric measurement of haemoglobin in unaltered whole blood and consists of photometer and cuvettes and that the cuvettes do not contain any reagents. The measurement used is the t-test method. Sorting the results into "Acceptable" and "Not Acceptable". A frequency table was made, the mean, standard deviation and confidence interval were calculated, and accordingly, a decision can be made.
Results: The study was done on random samples collected from 01/07/2016 till 28/02/2017. Out of a total of 508 samples, only 382 samples were found to have results that comply with Quality Monitoring results. The standard error was calculated as 0.019 and the 95% Confidence Interval ranges only between 71.4% and 79%.
Summary/Conclusions: There is significant difference between the test device results and reference quality monitoring results both clinically and statistically. It's recommended to consider a suitable corrective action to ensure having a reliable Haemoglobin measurement.

P-074

A COMPARATIVE ANALYSIS OF THE QUALITY OF QUADRUPLE BLOOD BAGS AT BLOOD TRANSFUSION INSTITUTE OF VOJVODINA

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Background: The quality of blood bags is a basic starting point in the chain for optimal blood product in every transfusion institution. Conditions to be met by manufacturers have eventually become very high and are controlled from internal to external eminent laboratories. Our Institute, which is the first in the country (from 1995 year) who got licensed under the ISO standards, during the annual announcement of the public procurement for the blood bags, requires the highest levels of quality, analogous to those in the EU. Nevertheless in the last 5 years we can see a significant difference in the quality of two manufacturers, both based outside the EU, but also with certificates of quality standards by European laboratories with the obligatory possession of the CE marking.
Aims: To compare and analyse the quality of blood bags used at Blood Transfusion Institute of Vojvodina.
Methods: We compare the two nine-month period in which we used solely bags of only one manufacturer: Since the beginning of August 2015 until the end of April 2016 - A bag manufacturers and the beginning of June 2016 to the end of February 2017 bags manufacturers B.
3 key quality parameters were monitored:

- The emergence of "modified blood" because of the inability to keep erythrocytes in saline-adenine-glucose-mannitol (SAGM) due to bursting of bags with SAGM. These red cells instead of 42 days have a shelf life 28 days (*Anticoagulant* Citrate Dextrose Solution – ACD-A)
- The second parameter is monitored blood bags malfunctioning in the process of making blood components
- The third parameter is accompanied by the technical errors that were created on automatic separators with open system, donated by the EU in the transformation process of blood transfusion services in our country

Results: In the period from August 2015 until the end of April 2016 (manufacturer A) we had 25 units of "modified blood," 74 malfunctioned bags and technical errors in 1,711 (taking into account any blood components: platelets, fresh frozen plasma and erythrocytes).
In the period from early June 2016 to the end of February 2017 (manufacturer B) we had 108 units of "modified blood", 148 bursting bags and 2,946 technical defects.
The number of blood units in the nine month period was almost identical (20,132: 20,201). Expressed as a percentage: with bags from manufacturer B we had an increase of "modified blood" from the 432%, increase in the number of bursted bags of 200%, a growth of technical errors amounted to 172.2%.
Summary/Conclusions: Despite the existence of the relevant written technical evaluation of accredited foreign laboratories, every country that wants to be completely sure that sought-quality requirements of the goods that are imported is fulfilled, should have its own top equipped laboratory for quality control of the goods or the National Agency before issuing a license for import goods requires at least another two independent appraisals of the quality by the world's accredited laboratory of its choice.

P-075

IMPLEMENTING THE QUALITY MANAGEMENT SYSTEM IN THE INSTITUTE FOR TRANSFUSION MEDICINE OF REPUBLIC OF MACEDONIA

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Background: The main aim of JACIE accreditation is to improve the quality of hematopoietic stem cell transplantation in Europe by providing a means that transplant centers, stem cell collection facilities and processing facilities can demonstrate high-quality practice. The Department for Stem Cell Collection is operational since 2001 year with 709 collections of stem cells (565 in patients and 144 in sibling donors) till 2017. This Facility is part of the Institute for Transfusion Medicine of RM (ITM). In 2012, in order to improve our overall service and to put it to the highest level, a quality management system (QMS) was developed.
Aims: Our purpose is to show our experience in implementing and maintenance of the quality management system and our first steps in achieving the JACIE accreditation in order to provide our patients and donors the best possible care.
Methods: We are implementing and maintaining QMS through the establishing of the ISO standardization for the whole institution (ITM). The two of our colleagues became the JACIE Inspectors and the standard operating procedures (SOPs) were developed, followed by regular meetings, trainings and self-evaluation of the personnel. We asked for the orientation visit from the independent JACIE inspector in order to come one step closer to the JACIE accreditation and to improve our overall QMS. We continue to work according to the quality management and improve our everyday service permanently.
Results: There is a national regulatory framework in place and WHO and World Bank initiatives in Macedonia which support quality in health care and accreditation. The Institute for Transfusion Medicine of RM was a part of the IPA project "Strengthening the Blood Supply System". This project aimed to ultimately bring the Blood Transfusion Service to European Union standards allowing the exchange of blood components and all other types of collaboration with other European Union countries in future. The project put the basis for unification of blood transfusion standards and operating procedures in the whole country as well as set up essential education of blood transfusion personnel. Document Management System was developed in order to improve our QMS. During the orientation visit from the JACIE inspector were found a lot of strengths in our QMS, but there are still a lot of areas for improvement. Our strengths are motivated team and supportive institutional leadership including Macedonian Ministry of Health. Areas for improvement are: labeling of cellular therapy products and lack of laboratory for quality control.
Summary/Conclusions: The requirement to demonstrate the quality of practice has currently become one of the main priorities for health care professionals in general. National authorities, funding foundations, health care organizations and medical industry increase pressure on clinical centers to demonstrate that clinical and laboratory practices are compliant with high quality standards of care. This required level of excellence can be demonstrated by means of accreditation. Our institution

has in plan to implement ISBT 128 standards for labeling of cellular therapy products and to establish a laboratory for quality control of cellular therapy, as well as to meet all the requirements to become JACIE accredited facility. In conclusion, working by standards, following the rules and regular self-evaluations will help us to maintain the strong quality management system.

P-076

ACCREDITATION OF BLOOD BANKS: EXPERIENCE AND RESULTS OF BLOOD BIOLOGICAL QUALIFICATION LABORATORIES, CENTER FOR VALIDATIONE AND PRODUCTION OF EMOCOMPONENTS (CPVE) IN TURIN, ITALY

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Background: Since year 2002, the most intensive and relevant as ever national and EU lawmaking activities in the field of blood transfusion were carried out, as well as activities associated with them.
The legislative framework that resulted is therefore quite complex in terms of relations between European and National legislation, in particular the need to harmonize the acts of European Directives with existing National standards.
In Italy the State-Regions-Agreement of 16.12.2010 defines the minimum structural-technological-organizational requirements for the pursuit of sanitary activity in blood banks and the verifying-procedures that must be apply in the processes for authorization and accreditation.
Aims: The goal of this work is to show the experience of Blood-Biological-Qualification BBQ in Turin accredited without any "non-compliance" with the intent to make available all the data we produced and to facilitate those who keep on working in this route.
Methods: Working-study group was created, the leader participated in regional meetings and courses for accreditation; a time-schedule was planned and all the 83 structural, technological and organizational requirements were analyzed; all the necessary requested evidences were produced. We set up specific procedures with the related operative instructions, documents, process indicators and targets. With respect to Validation master-plan we produced the "validation-document", the "risk analysis" and "instrument-qualifications" for BBQ process. We qualified also all the software used to manage the analytical results according our algorithms especially for the infectious diseases blood transmitted.
Results: After inspection-visit of Italian Inspectors for Accreditation, of EDQM inspectors and Kedrion inspectors, we were accredited with "non-compliance". The detailed results of all this huge work, tables, graphics, figures, and pictures (not allowed in this abstract) will be showed and discussed in the Congress.
Summary/Conclusions: This fatiguing, laborious route is very important and necessary for the blood banks because they are called to questioning the adequacy of its critical processes provided, the resources used in them, their technical-organizational planning skills to achieve quality objectives and aligned with the current regulations of EU.

P-077

INFECTION CONTROL SOLUTION FOR BEDSIDE BLOOD TRACKING DEVICES

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Background: Healthcare organisations have a responsibility to ensure that the risks associated with reusable medical devices are well managed in order to reduce risk of infection between patients and/or staff. Electronic systems for administration of blood components used at the patient bedside have been demonstrated to provide a safe solution for "right patient right blood" and cold chain issues and are becoming increasingly used in the healthcare setting. However, the devices commonly used, Personal Digital Assistant (PDA), present challenges in terms of infection control as they are used for more than one patient and do not tolerate excessive decontamination or sterilisation procedures.
During implementation of the Haemonetics BloodTrack Tx system, to reduce the risk of infection between patients and loss of PDA devices due to excessive cleaning a single-use, self-adhesive protective film (Packexe®) was introduced across all areas of the Royal Devon and Exeter NHS Foundation Trust involved in blood transfusion. This film covers the front of the PDA, is disposed after use and negates the needs for decontamination with excessive alcohol or other liquid cleaning substances.
Aims: To introduce a simple solution for infection control for point of care devices used for administration of blood components acceptable to local infection control procedures.
Methods: A self-adhesive protective film (Packexe®) of a suitable size was sourced, this film had previously been demonstrated to be effective at reducing contamination by up to 100% when used in Ablation or Therapy rooms and significant contamination reduction had been achieved during radioisotope treatments when applied to floors and surfaces. Smaller strips of the film were available to cover the PDA used in the administration of blood components.
Results: Since implementation of the protective film in April 2016 there have been no reports of transmission of infection between patients or loss of PDA devices due to cleaning procedures. The protective film has been demonstrated not to interfere with use of the PDA for electronic blood tracking or present difficulties for staff using the device.
Summary/Conclusions: The Packexe® single-use, self-adhesive protective film offers a simple solution for infection control for point of care devices used for blood component transfusion purposes. This solution has been accepted with this Trust as an effective infection control process and has been well received by clinical staff.

P-078

BENCHMARKING TO IMPROVE BLOOD ESTABLISHMENT PERFORMANCE

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Background: The primary aim of benchmarking is to improve one's own performances by comparing yourself with and learning from others who have achieved high standards of excellence.
Benchmarking in the health care sector is usually driven by efficiencies resulting in important cost-effectiveness, but patient outcomes and safety are always paramount.
Aims: Introduction of new evaluation concepts, improve the knowledge of the organization itself, identify areas for improvement, establish realistic objectives, create priority criteria in planning and learn from the best.
Methods: Given the participation of the Blood and Transplantation Centre of Coimbra in the EBA Benchmarking Group, it was possible to know the indicators used by the other participants and to establish targets for improvement in the processes of blood components preparation in our Centre.
All data concern the years of 2011 to 2014.
The indicators used and intention to improve were: percentage of unused blood components by shelf-life and percentage of components rejected in their preparation.
Results: The analysis of the data reveals the decrease of outdate of RC and pool of platelet during the four years analyzed from 3.4% to 2.8% in RC and 2.8% to 0.4% in pool of platelet.
The percentage of components rejected in their preparation was 0.23% to 0.28% in RC and 0.64% to 0.5% in pool of platelet.
It should be noted that due to the consolidation of blood components preparation in our Center, the average of components monthly prepared in 2011 were 7,106 RC against 11,796 in 2014 and 375 against 1,200 pool of platelets.
Summary/Conclusions: The major challenges that have been set before us include the definition of best practices and the development of cost-effective methods of data collection.
The findings show that data collected for benchmarking has the potential to be used for multiple purposes contributing to improved quality and efficiencies within our Blood Establishment.

P-079

COLLABORATIVE STUDIES TO CHARACTERIZE CBER REFERENCE PANELS FOR BLOOD GROUP GENOTYPING FROM A RENEWABLE SOURCE OF DNA.

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Background: Extended typing of red blood cells (RBC) is an effective strategy to reduce alloimmunization of patients who receive multiple transfusions, such as those with sickle cell anemia. Screening of large numbers of donors using traditional serological methods and matching for rare antigens is a resource intense activity often limited by the availability of reagents. Molecular blood group genotyping has been recognized as a reliable method that enables typing of blood groups that is high-throughput, multiplexed, and not affected by transfusion history or poor reactivity of serological reagents. Well-validated DNA reference panels are essential for the development, validation, manufacturing and quality control of the molecular genotyping assays. However, the availability of reference materials for blood group molecular genotyping is limited, which makes comprehensive coverage of many blood group systems difficult. Manufacturers of genotyping kits and genotyping laboratories use non-renewable clinical materials for reference, some of which are poorly characterized, increasing the probability of mistypings. Well-established reference panels will assist manufacturers in assay development and genotyping laboratories in test calibration and monitoring of performance.
Aims: To perform collaborative studies to establish renewable genomic DNA reference panels for molecular blood group typing assays, which will help improve genotyping accuracy and reduce probabilities of adverse events in blood transfusions.
Methods: Sample selection was based on blood donors' historical RBC phenotypes and DNA genotypes as determined by a collaborating blood establishment under an approved IRB. Over 30,000 donors were screened using a multiplex molecular assay, allowing selection of 53 donors carrying genetic variants of interest, covering polymorphisms in 41 target alleles associated with 17 blood group systems. Peripheral blood mononuclear cells were isolated and transformed with EBV to establish immortalized cell lines for use as renewable source of genomic DNA. Complete genetic characterization of each sample for each genetic polymorphism was performed using customized testing including 39 unique TaqMan allelic discrimination assays and 27 Sanger sequencing assays, covering multiple polymorphisms to determine and/or confirm the genotypes associated with RBC antigens of interest. For the formulation of panel, 18 cell lines that provide comprehensive coverage of desired alleles were selected, expanded, and DNA was isolated and lyophilized to ensure stability. Accelerated degradation and stability studies were performed. The candidate panel was distributed to 29 collaborating laboratories who agreed to participate in the studies for characterization of the first comprehensive reference materials for blood group genotyping. Participants included clinical and research laboratories and members of industry; they performed tests routinely used in their labs for genotyping and reported their results.
Results: Results were reported by 25 collaborators, who used a wide variety of methods, both laboratory-developed and commercial. Overall, there was a high level of agreement between collaborating laboratories on most of the genotyping tests requested. Genes that produced the highest levels of variation were FY, KN, OK, and RH. Errors, inconsistencies and procedural limitations of some laboratories indicate a need for validated reference reagents.
Summary/Conclusions: We have produced renewable reference panels for RBC genotyping and coordinated an international collaborative study to evaluate their performance.

Blood donation

Blood donor recruitment

P-080

A WEB-BASED APPROACH TO BLOOD DONOR MANAGEMENT IN HOSPITAL BLOOD BANK

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Background: Effective donor management is an important step to meet supply requirements in a hospital blood bank. Blood centers often experience irregular donor arrival rates leading to a stressful working situation for the staff and a compromised donor satisfaction due to long waiting times. The donation scheduling by fixed donor appointments may improve blood donation process. Capability of modern communication technologies has the potential to facilitate the visit arrangements. We have implemented a Web-based mobile application ("app") integrated with the hospital website to provide on-line donor appointments according to current hospital demand.
Aims: The aim of this study was to investigate the influence of regular on-line blood donation appointments on recruitment and retention rates among donors of different age and gender.
Methods: On-line donation appointments were planned for 3-5 donors per every 15 minutes interval according to blood bank supply requirements. We have compared donor distribution in age, gender, donation and retention rates before and after implementing the mobile donation scheduling. The data were observed during 9 month 2016 and compared with similar period in 2015 (before implementing the app mobile system).
Results: 2058 blood donors made 4762 on-line appointments during 9 month in 2016. Regular scheduled arrivals resulted in comforting environment both for blood donors and nursing staff. Empirically derived data revealed that population of young (< 20 years) fist-coming donors, mainly college students, increased by 9.5% in comparison with the same interval of 2015. However, donors older than 50 years were less enthusiastic to use mobile app – the rate of this group decreased from 5.2% to 2.4%. There was no significant difference in other age categories. The donation rate increased from 1.8 to 2.3 during the period. No difference in gender was observed.
Summary/Conclusions: The possibility of donation scheduling with on-line appointments was highly appreciated among blood donors. The use of mobile app leaded to enhanced recruitment and retention of perspective category of young donors with no difference in gender.

P-081

COGNITIVE PERSONALITY FACTORS INFLUENCE BARRIERS AND PERCEIVED EFFICACY TO DONATE BLOOD AND PLASMA

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Background: The choice to become a donor is personal but can be influenced by external factors (e.g. limited opening times) or internal/personal factors (e.g. attitudes or thoughts with regards to the donation procedure). Furthermore, donation barriers, attitudes and behavior may be dependent on certain cognitive personality factors, such as the way a person cognitively copes with negative emotions or adverse events.
Aims: To assess whether participants with certain emotion regulation strategies, approach/avoidance behaviors and state anxiety levels experience different barriers to blood and plasma donation, and whether these cognitive personality factors indirectly influence donation efficacy through the experience of these barriers.
Methods: The sample consisted of 619 participants from the general population (donors and non-donors) who participated in an ongoing study at the University of Chicago. They were asked to rate to what extent they agree with 25 possible donation barriers (on a 5-point Likert scale). Also, participants rated how capable they perceive themselves to donate (on a 5-point Likert scale). Additionally, they completed the State Trait Anxiety Inventory (STAI), the Emotion Regulation Questionnaire (ERQ), and the Behavioral Activation/Inhibition Scale (BISBAS). The ERQ measures cognitive reappraisal ("When I want to feel less negative emotion, I change what I'm thinking about") or emotion suppression ("I control my emotions by not expressing them") emotion regulation styles. The BISBAS measures the tendency to avoid negative stimuli or approach positive stimuli with four scales; Fun-Seeking, Reward Responsiveness, Drive ("I go out of my way to get things I want") and Inhibition ("I get worked up when something unpleasant is going to happen"). The 25 barriers were clustered using factor analyses. A model containing indirect effects of cognitive personality factors on self-perceived capability to donate, through the reported barriers clusters, was assessed with regression based path analyses.
Results: Four clusters of barriers were found: Distrust (i.e. the medical system), Fear (i.e. needles), Inconvenience (i.e. time-constraints) and Procedural Beliefs (i.e. discovering an illness). The Inconvenience barrier did not negatively impact efficacy, and participants with cognitive reappraisal strategies felt more capable to donate. However, participants with an emotion suppression style were more distrustful, fearful and worried more about the donation procedure. Anxious and Reward Responsive participants also tended to worry more, whereas inhibited participants were more

fearful. People who are very driven to reach their goals (BISBAS Drive) reported donation barriers most frequently. More importantly, the experience of these barriers affected self-perceived capability to donate: higher ratings of Distrust, Fear and Procedural Beliefs were associated with a decreased self-perceived capability.

Summary/Conclusions: Insight in what prevents potential donors from donation is key in developing efficient recruitment strategies. Different people experience different barriers, and may need a different information or recruitment approach. This knowledge could be implemented to develop personalized recruitment or information strategies, or retention strategies for first time donors. For example, as a cognitive reappraisal strategy is directly related to a higher, but emotion suppression to a lower, donation efficacy, this may prove to be a good intervention target.

P-082

INTRODUCING LOCAL HB SCREENING OF BLOOD DONOR APPLICANTS – COMPARING APPROACHES AND TECHNIQUES

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Background: Minimum required hemoglobin levels (Hb) for blood donors are 125 g/l for women and 135 g/l for men. Historically, a quarter of all women applying as new blood donors in Stockholm, and a tenth of men, do not fulfill this criteria. In Stockholm, Hb samples are routinely measured at a central hospital laboratory and applicants will in most cases not be informed of their Hb until the second visit.

Aims: This investigation examines two processes of local Hb-screening at blood donation, aiming at staff gaining confidence in capillary analysis at first visit for immediate dismissal of unsuitable donors. Also, compares two different Hb-instruments and capillary versus venous Hb concentrations.

Methods: Two 3-months studies were performed at one blood donation site in Stockholm, including actions to increase previous low trust for capillary testing, e.g. staff education, and staff involvement in establishing new processes.

First, local measuring of Hb for applicant blood donors was introduced. Venous samples were collected after completion of the donor health questionnaire (DHQ) and interview. Hb was measured locally on HemoCue Hb 201 DM Analyzer, and also in the central laboratory using Sysmex XE-2100.

Secondly, Hb was measured on HemoCue in a capillary sample before the donor completed the DHQ and interview, enabling immediate dismissal. If capillary Hb was above limit and the interview approved, a venous sample was collected and analysed in the central laboratory.

Surveys were used to evaluate staff experience of the processes.

Results: Applicants and staff appreciated direct result of Hb at the first visit. The staff valued to give advice at dismissal and was also motivated by time saving to screen Hb before going through the DHQ and interview process.

The mean difference in venous Hb concentrations when comparing HemoCue with Sysmex was −1.4 g/l (1.0%, n = 436), $P < 0.0001$, paired t-test, correlation (r^2) = 0.9045. The mean difference when comparing capillary samples measured on HemoCue with venous samples measured on Sysmex was 2.4 g/l (1.7%, n = 407), $P < 0.0001$, paired t-test, correlation (r^2) = 0.6852.

Hb below limit was found in 37%/26% women and 9%/14% men (first/second study). Capillary Hb-testing detected fewer women with low Hb (17%), with 9% of women with Hb below limit found only in the subsequent venous analysis. A majority (64%) of the dismissed women had Hb within normal range (117–124 g/l), and correlated with lower age.

Summary/Conclusions: Local Hb screening entails better donor service and early dismissal of donor applicants with unsatisfactory Hb. Implementation of local Hb analysis for donor applicants was successful. Capillary Hb-testing before DHQ and interview saves time for many women, but is for most men an unnecessary step.

The instrument bias was small. The higher Hb levels in capillary samples compared with venous samples has been shown by others and may have hemodynamic causes. The lower correlation when comparing capillary and venous Hb levels reflects a larger variation with capillary sampling, which was expected.

P-083

EFFECTIVE WAYS TO RETAIN FIRST-TIME BLOOD DONORS

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Background: Regular blood donors are the cornerstone of safe blood. Unfortunately most of the first time blood donors never return to donate again.

Aims: The aim of this field trial was to evaluate return rates of first time blood donors to donate again after different interventions.

Methods: This trial was conducted at 4 main blood centers in Iran. The studied population included first time blood donors who met blood donation criteria based on the national standards of Iranian Blood Transfusion Organization (IBTO), who were under 35 years of age, and whose results for routine screening tests (HBsAg, HCV Ab, and HIV Ag-Ab) were negative. Six different groups were assigned randomly based on the following interventions: (1) those that received reminder phone calls consequently 3 months after their first donation, (2) those that received an educational letter about the importance of donating blood 3 months after their first donation, (3) those that received an emotional letter about blood donation after 3 months of their first donation; they were then asked to return for another donation, (4) those that received a T-shirt at the end of the first blood donation in the donor recruitment department, (5) those that participated in a lecture meeting immediately following the first blood donation, and (6) those that received no intervention after blood donation as the control group.

The return rate of blood donors was defined as a second attempt to donate within 6 months after the first blood donation, irrespective of being eligible for blood donation or not. The return rates were calculated by using SPSS 16 software package (SPSS, Inc., Chicago, IL, USA).

Results: Out of 1,356 blood donor participants, 141 (10.4%) were female and 1,215 (89.6%) were male. 394 (29%) of the blood donors returned within 6 months for the second donation (95% CI: 0.26–0.31). The return rates for a second donation were classified based on the 5 different interventional groups. The return rates in the emotional letter group was 36% (95% CI: 0.31–0.42), in the educational letter group 33% (95% CI: 0.27–0.38), in the telephone reminder group 31% (95% CI: 0.25–0.37), in the incentive group 30% (95% CI: 0.22–0.38), in the control group 22% (95% CI: 0.17–0.27), and in the lecture meeting group 22% (95% CI: 0.17–0.27).

Summary/Conclusions: The study showed that the intervention with an emotional messages as the most effective intervention strategies to change the Iranian first time blood donors into regular ones. The incentive strategy was effective in donor retention, although it was the less effective intervention compared with letters or telephone reminders.

P-084

BARRIERS AND MOTIVATORS FOR GIVING BLOOD AMONG AFRICAN-SURINAMESE AND GHANAIAN PEOPLE; A QUALITATIVE STUDY IN THE NETHERLANDS

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Background: In many Western countries there is a shortage of blood donors from Sub-Saharan African background or descent. This restricts the diversity of rare blood types in current donor pools and may pose serious health risks for transfusion patients in need of these blood types.

Aims: The goal of this study was to acquire more knowledge in the barriers and motivators for donating blood among Sub-Saharan Africans in the Netherlands.

Methods: The study population consisted of first- and second generation migrants of two relatively large Sub-Saharan groups in the Netherlands: African-Surinamese (n = 20) and Ghanaian (n = 16) people. In personal, semi-structured interviews we uncovered their experiences and opinions regarding blood donation, barriers and motivators that could play a role, and we explored their ideas for donor recruitment. Interviews were transcribed and analysed in MAXQDA.

Results: Of the 36 participants, one Ghanaian man had ever donated in the Netherlands. Nine participants had donated in country of birth. Their main motivation for these past donations were incidental need or personal request at work or school. The Dutch blood bank organisation (Sanquin) was not known among the participants, but they did have knowledge and a positive attitude regarding blood donation in general. A few participants had a negative attitude regarding blood donation due to concerns with the safety of giving blood or due to religious beliefs (Jehovah Witness). The main reported barriers for giving blood were fear, in particular for

needles, losing too much blood, and health issues (HBV, stroke). The main motivators for giving blood were saving someone's life and being asked to donate. The participants reported that they would give blood if they knew they could help someone else by donating blood and were approached for it. But many participants also reported that the interview was the first time they heard of blood donation in the Netherlands and it was the first opportunity to think critically about giving blood. A personal approach, in which information is provided on the process of blood donation and the need of rare blood types, was commonly considered to be a good recruitment strategy. The person to give this information would preferably be an influential person within the community, or someone with a lot of knowledge about blood donation, such as staff members of the blood bank or a general practitioner.

Summary/Conclusions: Awareness of the blood bank organisation and fear for blood donation seem to play an important role in the lack of blood donation among Sub-Saharan Africans in the Netherlands. Recruitment of this group should focus on increasing visibility and knowledge about the process of blood donation, and emphasising the need for specific blood types.

P-085

EVALUATION OF IRON STORES IN CROATIAN BLOOD DONORS

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Background: With each donation of whole blood, healthy blood donor loses 200–250 mg of iron. This loss is compensated by the mobilization of stored iron. Several studies showed that blood donors may be at risk of iron deficiency/depletion and the development of anaemia. This risk is particularly pronounced in female donors of childbearing age and frequent blood donors. Therefore, implementation of numerous measures is considered in order to prevent iron deficiency in blood donors. Determination of ferritin is the most widely used method for estimating iron stores in blood donors.

Aims: The aim of this study was to evaluate iron stores of voluntary blood donors in Croatia, by measuring ferritin level in donors with different donation frequency in two years preceding the inclusion in the study (0–8 for man and 0–6 for women), and to consider the implementation of additional measures to prevent iron deficiency, particularly the introduction of ferritin testing in blood donors.

Methods: The study involved 1,084 male and 792 female whole blood donors, eligible for blood donation, including requirement for the minimum haemoglobin concentration (125 g/l females and 135 g/l males), estimated by the copper sulphate method. At the beginning of blood donation, additional sample was collected from the sampling bag for the determination of serum ferritin. Serum ferritin was measured on Cobas c311 (Roche Diagnostics, USA), using Roche FERR 4 Tina-Quant. Statistical analysis was performed using the MedCalc software and Excel 2015.

Results: In male donors, serum ferritin decreased linearly with increased frequency of blood donations: from the median of 129 μg/l in the control group to 43 μg/l in those with 7–8 donations in two years. In control group of female donors, the median value of ferritin was 33 μg/l, while median ferritin in test groups was within a narrow range between 22 μg/l and 29 μg/l, and in correlation with the menstrual status. Depleted iron stores (ferritin <12 μg/l) were found in males with the frequency of 0% in the control group and 1.95% in those with 1–8 donations in two years. Depleted iron stores were found in 6.7% females of the control group and in 17.2% of female donors with 1–6 donations in two years. Linear regression analysis confirmed that the frequency of blood donation is strong negative predictor of ferritin concentration ($P < 0.001$) in both, man and women (standardized beta coefficient −0.60 for man and −0.36 for women).

Summary/Conclusions: Despite numerous studies published so far, the risk of iron deficiency in blood donors is still a topic of ongoing interest. The experiences of different countries are important because of the differences in the structure and characteristics of the donors, dietary habits of the population and different criteria related to the allowable volume and frequency of blood donations. In addition to more intense education of our blood donors on the importance of iron and its loss during the blood donation, we propose the replacement of copper sulphate test with quantitative method of haemoglobin determination, along with the gradual introduction of ferritin determination.

P-086

RETROSPECTIVE ANALYSIS OF CAPILLARY HEMOGLOBIN RECOVERY IN NEARLY 1,200,000 BLOOD DONOR RETURNS

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Background: The Finnish Red Cross Blood Service is a national blood establishment that collects, processes, and distributes all donated blood in Finland. As part of the standard procedure before blood donation the concentration of capillary hemoglobin (cHb) is measured.

Aims: The aim of this study was to retrospectively assess the time period needed for cHb recovery after blood donation in Finnish blood donor population.

Methods: We utilized data mining tools for a large, retrospective data set of all 1,163,524 donor returns that took place in Finland in 2010–2015.

Results: The results show that the average recovery times for cHb to return back to the level of preceding donation were substantially longer, over 200 days in all age-groups, than the minimum allowed donation intervals. cHb recovery was especially poor in women under the age of 30 who returned to donate soon after the minimum allowed donation interval. Frequent donors recovered substantially faster, with the average recovery times of ~100 days in men and ~200 days in women, than infrequent donors, suggesting that there is a subpopulation of donors who can donate frequently without fear of iron deficiency. Return interval in fact explained only 1% of the variation in cHb recovery, which points to unknown, individual features, such as genetic or lifestyle factors, warranting further studies and suggesting that simply extending the allowed donation intervals may not suffice to improve cHb recovery.

Summary/Conclusions: The study demonstrates that data mining of blood establishment records is a powerful tool for depicting features of blood donor population.

P-087

THE INFLUENCE OF TELEPHONE CONTACTING ON THE RETURN OF FIRST-TIME DONORS

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Background: In Finland, Finnish Red Cross Blood Service is the only provider of blood products for 5.5 million population. There were approximately 200,000 whole blood donations in 2016. Of the total of 132,000 donors, 18,000 were first-timers.

In Turku and Seinäjoki donor centres from January to July 2015, there were 672 (6%) first-time donors, as the total of donations number was 11,816. In these centres during same time period in 2016, there were altogether 11,438 blood donations. Of these, the number of first-time donors was 677 (6%).

Aims: Our aim was to study the influence of personal contact by telephone and its effect on return of first-time donors.

Methods: From January to July 2016, we asked the first time donors for permission to contact them by telephone or text message (SMS), in order to inform on their ABO blood type. If the donor was not reached by telephone, a SMS was sent with a toll-free donor helpline number for inquiry about the blood type.

We collected data prospectively on the return of first time donors from until December 2016. The data of the corresponding time in 2015 was obtained from our online reporting system (QlikView®). We analysed how many of the first-time donors who were contacted by telephone or SMS, returned within 6 months, compared to first-time donors from the same time period, who were not contacted. The return rate was also compared with the data from the previous year, when no contacting of first time donors was done.

We used to two-by-two tables to evaluate the association between contacting by telephone or SMS and its effect on return of first-time donors.

Results: In 2015, 199 (30%) of the 672 first time donors from January-July returned by the end of the year. In 2016 altogether 220 (32%) of 677 first-time donors from January-July returned by the end of the year 2016.

During January-July 2016, we contacted 243 first-time donors by telephone or SMS, 109 (45%) returned by the end of the year, compared with 111 (26%) of the first time donors not contacted. The result is statistically significant (OR 2.4, CI 95% 1.7–3.3).

Summary/Conclusions: Only 243 (36%) of the first-time donors gave us a permission to call. It would be important that nurses looking after the first-time donors remember to ask for the permission to call after blood donation. It is essential that nurses understand the importance of the retention of the first-time donors.

P-088

ELIGIBILITY ASSESSMENT AND WILLINGNESS TO REGISTER AS A BLOOD DONOR OF MEN WHO HAVE SEX WITH MEN

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Background: Several Blood Services have recently changed, or are currently reevaluating their blood donor policies for men who have had sex with men (MSM). It is plausible that more countries will adopt a less strict deferral in the future, given that screening methods have improved, and awareness and knowledge regarding HIV and other blood transmissible infections have increased. Few studies have assessed the consequences and opportunities for recruitment.
Aims: In this study, we aimed to assess which proportion of MSM would be eligible to register as a donor, given their reported history of male-to-male sex and other deferrable risk behavior. In addition, we investigated if (eligible) MSM were willing to register as a blood donor.
Methods: We invited members of a research panel (n = 4,422) who were eligible to donate blood in the Netherlands according to their age (18–69 years old) to participate in an online survey. We assessed eligibility to donate blood by asking questions about intravenous drug use, receiving money in exchange for sex (both criteria for permanent deferral), paying money in exchange for sex, having sex with an intravenous drug user, having sex with a person who is infected with HIV (all three criteria for 12-months deferral), and male-to-male sex. Furthermore, we asked if participants were willing to register as a donor.
Results: The overall response rate was 60% (n = 2,654). Two-hundred and thirty men (8.7%) reported that they ever had male-to-male sex, and that they were not registered as a donor. Of these MSM, 34.3% reported that their last male-to-male sex was over a year ago. According to their reported risk history and last male-to-male sex, 32.2% of these MSM would be eligible to donate within a 12-month deferral policy. In other scenarios, 42.6% (4-month deferral), 38.7% (6-month deferral), and 18.7% (5-year) of MSM would be eligible to donate blood. Of MSM who were eligible according to their reported risk history, regardless of their last male-to-male sex (n = 203), 47.8% reported a moderate/high willingness to register.
Summary/Conclusions: A 12-month deferral after the last male-to-male sex is a widely used criterion by Blood Services. About one-third of MSM in our study would be eligible to register according to their reported risk history in this scenario. Higher proportions of MSM would be eligible to register in shorter deferral scenarios. Almost half of MSM who would be eligible, regardless of their last male-to-male sex, reported a moderate/high willingness to register as a donor. These results show that MSM are interested in donating, and could therefore potentially be recruited to replenish the declining male blood donor pool in the Netherlands.

P-089

"PROGETTO CUORE": SOFTWARE FOR ASSESSMENT OF CARDIOVASCULAR RISK IN A COHORT OF BLOOD DONORS

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Background: Nowadays the cardio and cerebrovascular diseases are in Italy one of the most important problem for the public health and are also the fundamental cause of morbidness/morbidity and disability.
Aims: The Transfusion Center of Aversa in order to evaluate the relation between the cardiovascular risk factors and unhealthy lifestyles, to estimate the prevalence of risk factors (hypertension, dyslipidemia, overweight/obesity, diabetes) and to monitor over time the trend of these risks factors, has established a cardiovascular screening program called "cardiorisk" reserved to blood donors.
Methods: The "cuore.exe" program is a software that you can download via the following website www.cuore.iss.it which allows you to estimate the probability of experiencing your first cardiovascular event in the next 10 years. The global absolute risk has been calculated using the individual score of "Progetto Cuore" which is based on the measurement of the following eight variables: gender, age, smoking status, total cholesterol, HDL cholesterol, glycaemia, blood pressure, antihypertensive therapy. On the basis of the final score have been created three risk classes:

- high (greater than or equal to 20%);
- risk to keep under control through the adoption of healthy lifestyle (more than or equal to 3% and less than 20%);
- low risk (less than 3%).

Results: From January 2016 to January 2017 have been conducted 1.600 evaluations thanks to the cuore.exe software. The age range of donors enrolled at the program is 35–69. The research has found that the average cardiovascular risk in women, aged between 35–39 and 40–49 is less than 3%, 0.8% and 1.8% respectively; on the contrary for the men the risk is higher than 3% already between 40 and 49 years (3.5%). The risk for the women between 50 and 59 however is higher than 3% (3.2%) and it reaches 18.8% at the age of 60–69. Concerning men aged 50–59 the cardiovascular risk is around 10% that reaches up 17.7% at the age of 60-69.
Summary/Conclusions: "Progetto Cuore" was found to be an effective predictive medicine instrument, in fact have been implemented simple interventions aimed to improve the donors' s lifestyles. Special emphasis has been given to blood pressure measurement, the abolition of smoking, good nutrition and to the increase of physical activity in order to control the body weight and to have good health conditions over time. The widespread dissemination of this cardiovascular screening model related to blood donation is not only having an important impact in terms of public health but it has also demonstrated to be a good incentive to recruit new donors.

P-090

VOLUNTARY NON-REMUNERATED BLOOD DONOR TRENDS FROM BLOOD DRIVES IN KARACHI, PAKISTAN-SINGLE CENTRE EXPERIENCE

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Background: Blood supply in Pakistan is mostly dependant on replacement donors, provided by the patient's family and friends. To shift the paradigm towards voluntary blood donations, the first centralised blood centre of Pakistan (The Indus Hospital Blood Center – TIHBC) was established. It began conducting blood drives across Karachi and its suburbs.
Aims: To determine the trends of voluntary blood donations in the different organisational sectors of Karachi, Pakistan, along with donor demographics and seasonal variations.
Methods: Data was extracted from the Blood Drive and Donor Selection Modules of the Blood Bank Management Information System (BBMIS) database for the period of 37 months (December 2013 till December 2016). Data was analysed to identify major organisational sectors contributing to voluntary donations.
Results: Total 931 blood drives were conducted in 435 organisations, comprising of 308 (33%) factories, 205 (22%) corporate offices, 144 (15%) higher educational institutions, 118 (13%) sites of worship, 77 (8%) religious seminaries (madrassas) and 79 (9%) miscellaneous sites. In these blood drives, 54,292 volunteers were screened and 37,240 (69%) were drawn, including factories 15,752 (42%), higher educational institutes 8922 (24%), corporate offices 6151 (17%), religious seminaries (madrassas) 2769 (7%), sites of worship 1656 (4%), miscellaneous sites 1990 (5%). Thus, on average 51 and 62 donors per drive were drawn from factories and educational institutions, respectively.
Over the 3 years of the holiday season of the entire month of Ramadan, a total of 144 blood drives were conducted, comprising of 110 (76%) sites of worship, 27 (19%) religious seminaries (Madrassas) and 6 (4%) residential areas. Blood collection was performed after night prayer gatherings as people were fasting during the day.
The mean age of voluntary donors from educational institutions and religious seminaries (Madrassas) was 24 years while from all other sectors, the mean age was 30 years.
A total of 2,254 (6% of the total collection) female's donors were drawn, of which 1,288 (57%) belonged to higher educational institutions.
Summary/Conclusions: Factories were the highest contributor to blood collection. Even though fewer blood drives were held in educational institutions, they were the second highest contributor to blood donor pool. During Ramadan, blood was collected from sites of worship and religious seminaries (madrassas) only.
Males are the main contributors to voluntary non-remunerated blood donations. Furthermore, educational institutions contributed to most of the blood collected from females. Mean age of blood donors was lower in educational institutions compared to all other organisational sectors.
The future marketing strategy should be based on focusing the educational and industrial sectors as they are likely to offer more donors with lesser drives and efforts. Similarly, in Ramadan, sites of worship should be targeted to conduct blood drives. Females need to be mobilized towards voluntary blood donations.

P-091

TRANSFUSION SAFETY: COLLABORATION BETWEEN BLOOD TRANSFUSION SERVICE AND VOLUNTARY ASSOCIATIONS

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Background: The role of Transfusion Service is to produce safe and effective blood products; for this reason it is crucial collaboration with voluntary associations. Therefore, for this reason, it is drawn up a weekly program for the collection of blood, based on the regional and national self-sufficiency level because the real need for blood products cannot yet be satisfied with occasional donations, which are less controllable, non-programmable, with potentially higher risks for both the donor and the patient. Voluntary associations have devised, in this regard, various strategies to encourage the loyalty of donors, among them: Social Networking, Blogging, Campaign for the Prevention cardiology, andrology, thyroid and nutritional benefits Events and Awareness in schools.
Aims: Aim of the study is to analyze the change in the number of occasional and regular donors over the years, and how the awareness campaigns have been useful.
Methods: It is been evaluated, by the management system Eliot Health Engineering, the number of periodic and new donors from 2009 to 2016, calculating the percentages, the annual change and positivity to virological tests.
Results: 2009 periodic donors: 50; % Periodic donors: 6; New donors: 770; % New donors: 94; Positive donors to virological tests: 2.
2010 Periodic donors: 350; % Periodic donors: 7; New donors: 4,504; % New donors: 93; Positive donors to virological tests 1.5
2011 periodic donors: 1,462; % Periodic donors: 18; New donors: 6,757; % New donors: 82; Positive donors to virological tests: 1.4
2012 periodic donors: 2,113; % Periodic donors: 21; New donors: 4,848; % New donors: 79; Positive donors to virological tests: 1.6
2013 periodic donors: 2,761; % Periodic donors: 25; New donors: 8,501; % New donors: 75; Positive donors to virological tests: 1.6
2014 Periodic donors: 3,327; % Periodic donors: 27; New donors: 8,448; % New donors: 73; Positive donors to virological tests: 1.3
2015 Periodic donors: 4,268; % Periodic donors: 26; New donors: 11,905; % New donors: 73; Positive donors to virological tests: 1.1
2016 Periodic donors: 4,255; % Periodic donors: 25; New donors: 13,024; % New donors: 75; Positive donors to virological tests: 1.2
Summary/Conclusions: The active collaboration between ST and voluntary associations has allowed the significant increase in regular donors from 6% to 27%, with annual 4% increase. In contrast, the positive donors to virological tests decreased from 2% to 1.2%. This shows how the strategies implemented with awareness have been effective not only to increase the number of donors but also the quality of the product. This is our main goal, which also contribute voluntary associations, in order to ensure to patients who need it, an adequate transfusion therapy, safe and quality, thanks to the contribution of periodic donors, volunteers, anonymous, self-employed, managers, associates. In conclusion, we observed that an active and effective cooperation between the transfusion service and voluntary associations is crucial to ensuring the protection of donors and patients.

P-092

EVALUATION OF A POLICY FOR RETESTING THE DEFERRED DONORS HEMOGLOBIN: A PILOT STUDY

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Background: Low donor Hb accounts for half of deferred donors in our setting. With the introduction of the Hb 201 hemoglobin testing system in our donor selection protocol, we planned to study the impact of using this new donor Hb testing method on preventing unnecessary donor deferrals due to inappropriate Hemoglobin (Hb) testing results by the currently used method.
Aims: To compare the performance of the $CusO_4$ method with the Hemocue (Hb 201) method & evaluate the impact of a new strategy to retest the Hb of donors (deferred by $CuSO_4$ method) on recruitment of eligible donors.
Methods: The hemoglobin of deferred blood donors was retested using a capillary blood sample with Hemocue and the blood donation was accepted when the Hb was greater than 12.5 g/dl. Hb results with calibrated automated cell counter using venous blood sample were considered as standard reference value.
Results: Hb of 3,210 deferred blood donors was re-evaluated. The sensitivity, specificity, PPV & NPV of Hemocue method was better than the CuSO4 method with both capillary & venous samples. Bland & Altman plots showed that there was a good agreement between the Hemocue & reference method using the capillary (Avg. difference = 0.38 & $r = 0.86$) and venous blood samples (Avg. difference = 0.69 & 0.81).
With the new strategy, 27% of the deferred donors could be accepted back for blood donation. In 3% of these donors, the venous Hb by the standard reference method was found to be less than the recommended donor Hb level of 12.5 g/dl. Moreover, Hb values of 15% of the deferred donors were between 12 and 12.4 g/dl with the reference method.
Summary/Conclusions: The Hemocue (Hb 201) can be used reliably and safely with capillary samples for donor hemoglobin estimation. The Hb retesting strategy helps in recruiting additional eligible donors for blood donation. This re-testing approach may prove to be a useful strategy for prevention of unnecessary donor deferrals in the resource constrained setting. A lower threshold of 12 g/dl would result in increased acceptance of eligible donors for blood donation and therefore needs to be seriously considered in our country.

P-093

Abstract has been withdrawn.

P-094

TRENDS OF MAJOR DONOR DEFERRAL REASONS DURING THE PAST ELEVEN YEARS (2006–2016)

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Background: The safety of the blood supply is ensured through several procedures from donor selection to testing of donated units. Examination of the reasons for donor deferral can provide insight into the role that deferrals play in transfusion safety and helps in planning more efficient recruitment strategies and evaluating donor selection criteria.
Aims: This study aimed to investigate the trends of donor population and major deferral reasons during the past eleven years (2006–2016) to obtain basic data to be used in policy formation for donor selection and donor management for retaining donor pool.
Methods: The data available as donor deferral record over a period of 11 years from 2006 to 2016 was obtained and analyzed by searching the Blood Information Management System (BIMS) and Annual reports of the Korean Red Cross.
Results: Overall deferral rate has continuously decreased from 22.5% (the highest in 2007) to 14.8% (the lowest in 2015). Temporary deferral was more common than permanent deferral. Causes among deferral were anemia including low blood specific gravity (SG), past reactive test result, general health status, medication and health history for the last one year. More than 90% of those deferred due to anemia were women.
Summary/Conclusions: The pattern of donor deferral identified is an important tool for blood safety and also provides key areas to focus on a region or policy formulation nationally for donor selection as well ensure donor safety.

P-095

Abstract has been withdrawn.

P-096

OUTCOME OF THE INTRODUCTION OF THE PROTOCOL OF READMISSION OF DONORS WITH FALSE POSITIVE RESULTS FOR SEROLOGICAL TESTING FOR HBV, HCV AND HIV

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Background: The reactivity of serological tests for HBV, HCV, HIV1/2, in presence of negative NAT is commonly found in the daily activity of a transfusion center.

Such reactivity, in a significant number of cases, is configured as non-specific and linked to factors not known in part, leading then to the suspension of a large number of donors. Reactivity may be regarded as false positivity after the application of a readmission protocol providing adequate confirmation tests and a suspension and follow-up period during which the donor is checked through alternative serological tests. The false-positive serological test is fostered by some level of inevitable uncertainty of screening tests for the preference given, in the context of transfusion, to sensibility instead of specificity.
Aims: Estimation of the number of donors readmitted to the donation by the introduction of the readmission protocol.
Methods: For donors readmission we applied, starting from January 2015, a protocol that provides for the recall of the donor within 7 days after the reactive outcome of the test and the carrying out of subsequent checks on the 3rd and 6th month after, using a serological alternative test of comparable sensitivity and verifying the constant negativity of the donor with confirmatory testing and NAT performed on a single sample. The donor is readmitted at the end of the follow-up period if all the tests performed during the control period are negative.
Results: At the transfusion center ASL Caserta, from January 2015 to December 2016, we enrolled a total of 168 donors with reactive results: 97 for HBsAg, 53 for Anti-HCV and to 18 for Anti-HIV. Of these donors, 91 have completed the follow-up and were readmitted to the donation, 56 left the follow-up after the first initial check (as there were no subsequent checks), 11 are currently in the period of follow-up, 10 were suspended definitively for reactivity of the alternative test.
Summary/Conclusions: The application of the readmission protocol to donors with serology reactive tests allowed us to readmit 91 donors so far and to deal with the problem of the communication to the donor of the reactivity of virological tests in a structured way, allowing for a more serene dialogue with the donor, based on the certainty of a well-defined path and a potential readmission. If we consider the perspective of a systematic and extensive adoption of the protocol, we have also to recognize the importance of the reduction of the number of suspended donors with obvious consequences on the number of taken and available blood units.

P-097

THE EFFECT OF WORLD BLOOD DONOR DAY ON DIGITAL INFORMATION SEEKING AND DONOR RECRUITMENT

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Background: The purpose of World Blood Donor Day (WBDD) is to raise awareness for the importance of unpaid and voluntary blood donation.
Aims: The aim of this study was to quantify the impact of WBDD on digital information seeking on "blood donation" and donor recruitment.
Methods: Google trends data were used to quantify seeking behaviour on "blood donation" and "blood donor". The relative search volume (RSV) expresses the proportion of searches for term of interest amongst all Google searches for a specific timeframe and region. Differences in RSV between the period of interest and the control period were calculated. The period of interest was defined as 3 weeks surrounding WBDD. The control period consisted of the rest of the year, excluding August 2016 (Missing Type Campaign) and a wash out period of 2 weeks after the campaigns. Also, we compared mean difference in RSV to assess the additional effect of hosting WBDD using translation of the search terms into the languages of the countries which hosted WBDD in the last 6 years. Thirdly, web analytics were used to compare the period of interest with the control period, with respect to page views of the Sanquin website and new Facebook likes. Finally, the number of newly registered donors in June 2016 was compared with the average number of newly registered donors per month in the rest of the year.
Results: The average RSV for "Blood donation" in the period of interest was 78.6, compared to 72.1 in the control period (mean difference 6.5; 95% confidence interval (95% CI) 1.2–11.8). For "blood donor" this was 78.9 compared to 65.9 (difference 12.9; 95% CI 8.1–17.8). We found no additional effect of hosting WBDD. In the period of interest, the website of Sanquin was visited on average 6,862 times a day and 4,293 times a day in the control period (mean difference 2,569 (95% CI 1,687 to 3,451). In June 2016, 2,110 (95% CI 116 to 4,104) extra new donors were registered compared to the average 3,866 per month in the rest of the year. This represents an absolute increase of 54.6% (95% CI 53.0–56.2).
Summary/Conclusions: An international campaign like WBDD raises the awareness of blood donation and is effective in convincing people to become a blood donor.

P-098

THE MOST COMMON REASONS FOR DEFERRAL OF BLOOD DONORS IN INSTITUTE OF TRANSFUSION MEDICINE OF FEDERATION BOSNIA & HERZEGOVINA

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Background: Blood donors are for various reasons deferred and excluded from donating for a shorter or longer period of time. Reasons for the deferral of blood donors varies from center to center, but all our reasons are based on the recommendations of the World Health Organization (WHO).
Aims: We want to analyze the most common reason for deferral as well as the age groups and gender that are affected by the deferral in the Institute for Transfusion Medicine of FBiH in Sarajevo.
Methods: We did a retrospective analysis of the records of donors, from 01 October 2014 to 23 November 2016. Each donor has been viewed by the general practitioner. Based on a brief physical examination, blood pressure, hemoglobin, it is estimated if the blood donor is capable for blood donation. In case of deferral, reason for deferral is stored in the information system Wizard. From the base of reasons for deferral, deferred donors we sort by cause, gender, age group.
Results: In accordance with WHO recommendations, we have identified the three most common reasons: inadequate hemoglobin, low body weight and inadequate blood pressure. In a period of 26 months at the Institute for Transfusion Medicine of FBiH in Sarajevo we registered 41,391 voluntary donors. Women accounted for 39.5% of registered donors, and man 60.5%. From that number of deferred blood donors is 8,550 (20.6%) for various reasons. The number of deferred male was 9.26% and 11.38% of women. Low hemoglobin, low body weight, and hypotension made the reason for the 41.5% of deferred donors.
The highest rate of deferral was in the age group of 18–26 years, and the most common cause is low hemoglobin level. The most common cause for temporary deferral in female donors is anemia, in men this is low weight.
The blood donors from 18–26 years of age in our Institute makes 48.8% of the population of blood donors. This is also the most vulnerable populations, given that the rate of deferral in this population is 28.1%.
Summary/Conclusions: The largest number of deferral is in the group of first time registered blood donors, them 61.5%, other 38.5% is from group of multiple blood donors.
Our policy of promotion blood donation is based on the animation a large number of young people, with the conscious risk that many of them will be deferred. Registering new young donors make the new donor base, but also recommend need for a new strategy care for young generation of blood donors.

P-099

ANALYSIS OF BLOOD DONORS IN A TERTIARY CARE HOSPITAL AS A FIXED COLLECTION SITE

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Background: Bleeding or blood transfusion therapy is an important factor in the treatment of patients with anemia; thus, supply of blood components should be stable prior to transfusion. For a reliable blood supply, efforts should be made to increase donation rates. To increase the convenience of blood donation, a particular area should be designated as a fixed blood donation site, as this can have a significant effect on the rate of increase in blood donation.
Aims: This study analyzed the characteristics of blood donors at blood donation center in hospital and efforts to increase blood donation rate. Our finding could be useful for increasing blood donation at blood centers in hospital.
Methods: We retrospectively reviewed 687 blood donations including whole blood donation at a blood center in Pusan National University Yangsan Hospital (PNUYH) from March 2011 to June 2016. Repeated donors were included. Donor characteristics, including sex, age, address, and profession was recorded. Address were collected from the questionnaire of donation. Donors filled the address of residence or working place. Data of 2014 Korean Red Cross Annual Report were evaluated to assess the effect of convenient accessibility on donation rate. To calculate distance from the high school or college to blood collection center, information of school were obtained from website

of Busan metropolitan city office of education. Distance between each school and blood collection center were calculated using a Google map.
All analyses were performed using SPSS v.21.0 (IBM Corp., Armonk, NY). Chi-square tests, Fisher exact tests and Mann-Whitney U-tests were used for intergroup comparisons. A P value <0.05 was considered significant.
Results: A total of 687 blood donation were performed in this study. The majority of donors (65.2%) were aged between 20 and 30 years and female donation rates were 17.9%. The initial donors accounted for 83.7% (575 donations). There were 67 repeated donors (173 donations), who donated an average of 2.6 times including initial blood donation. Details regarding the residence of the donors, distances from blood donation centers, the most frequent donors were high school or college students and residents within 4 km radius of the blood donation centers. The farther away from the blood donation center, the more office workers and the business owners were the most frequent donors. The office workers and the business owners were act around the work place, therefore the address of home do not correlate with the distance between blood donation centers and address.
The association between blood donation centers and the presence of high schools or colleges according their distance between them were evaluated using 2014 Korean Red Cross Annual Report. The percentage of teenage donors and donors in their twenties are significantly differ according to the presence of a college within 4 km radius of the blood donation center (P = 0.03). Distribution of high school did not differ between the regions, percentage of teenage donors are not significantly differ according to the presence of a high school.
Summary/Conclusions: Although we do not effort to advertise our blood donation site actively, the blood donations increased. Our findings are the analysis of a single institution, it is difficult to generalize. Further studies including surveys about behaviors of blood donors are necessary. To recruit young donors from areas where there are no fixed blood donation center, a regional hospital can be used as a blood collection site.

P-100

PREVENTIVE LABORATORY CHECK-UPS AMONG BLOOD DONORS FOR BETTER BLOOD DONOR'S SAFETY

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Background: Blood donor selection is the first step in blood donations process. Any tool that can help in better follow-up of the health status among blood donors is cost-beneficial for donor itself and the blood bank. Within the National Institute of Transfusion Medicine (NITM) in Skopje, since the year 2010, there is a possibility for blood donors, on a voluntary basis, to make a laboratory check-up for biochemical analyzes, such are: cholesterol, triglycerides, glycemia, urea, creatinine, iron, ALT and AST. In this way, Institute provide free of charge preventive analyses and regular health check –ups for blood donors, and additional control in post-donation process.
Aims: To analyze the results of a preventive laboratory check-ups among blood donors at NITM in Skopje, to see the levels of biochemical values and number of blood donors that were included in post-donation counseling due to higher or lower laboratory values associated with some health risks.
Methods: By retrospective study were analyzed data from electronic evidence of performed laboratory tests for 4 parameters: cholesterol, triglycerides, glycemia and iron, in three months period (January-March, 2016). The gained results were statistically analyzed for maximum, minimum, average values and standard deviation.
Results: At NITM in Skopje, during the year 2016 were realized 22.934 blood donations. The biochemical tests were performed for 962 donors (4.2%). For the aim of this study, were analyzed data of 320 blood donors (33.3%). The maximum level of triglycerides was 10.1, minimum 0.1, average 1.5 and SD 1.43 ± 1.49 (reference values: 0.2–2.3); the maximum level of cholesterol was 16.3, minimum 2.2, average 6.7 and SD 1.89 ± 6.72 (reference values: 2.0–5.5); maximum glycemia level was 17.2, minimum 3.1, average 4.8 and SD 1.44 ± 4.75 (reference values: 3.5–6.5); the maximum level of iron was 41.9, minimum 1.8, average 19.8 and SD 4.66 ± 19.79 (reference values: 7.1–28.6). The biggest number of donors have high values for cholesterol (233 or 72.8%), high triglyceride levels (43 or 13.4%), high glucose level (24 or 7.5%) and (2 or 0.6%)of blood donors are with low iron levels. The post donation counseling was performed for all blood donors with values that differed from reference ranges of the analyzed biochemical parameters (302 or 94.4%).
Summary/Conclusions: Those laboratory results have helped to discover some medical conditions that were unknown for blood donors before. From the abnormal values of analyzed samples can be concluded that additional study should be performed in order to evaluate the factors, that may have influenced the test results. The donors are satisfied with the possibility to make free of charge biochemical analyses and then to discuss with physicians from NITM about the healthcare risks. The authorities from the NITM by implementation of this additional laboratory analysis have showed a blood donor oriented care, as well as increased measures for better retention, follow-up and donor awareness of dietary habits before blood donation. Due to this study were prepared brochures for healthy nutrition.

P-101

APPLICATION OF SOCIAL MEDIA IN THE MANAGEMENT OF APHERESIS PLATELET DONORS

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Background: With the increasing need for blood in clinic in China, the imbalance between blood supply and demand in China is becoming seriously. Social media such as Facebook has been used for blood donor's recruitment with a matching blood type through posting urgent messages in some countries. WeChat is the most popular social media in China, which has hundreds of millions of active users with a mature operational mode. Therefore, we use the WeChat platform to promote the recruitment of apheresis platelet donors.
Aims: To use WeChat platform for recruiting the apheresis platelet donors and explore proper ways to collect more apheresis platelet and save more lives.
Methods: Firstly, established and launched the "Beijing Apheresis Platelet Donation" WeChat platform in May 2016. Secondly, to increase the followers of the WeChat public number through a series of activities. For example, hold a series of on-site activities, and encourage participants to scan the QR code to follow "Beijing Apheresis Platelet Donation" WeChat; post voluntary apheresis platelet donation information on the WeChat platform which could be forwarded in the WeChat Moments by enthusiastic fans, so the number of followers can increase quickly. Thirdly, to encourage the WeChat follower from the potential to real apheresis platelet donors through routine interactions in WeChat platform. Finally, apheresis platelet donors can enjoy exclusively various member online and offline activities and achieve more high-end blood donation service through setting up a membership system.
Results: The number of apheresis platelet donors was increasing in 2016. Compared to 2015, the total number of apheresis donors was increased by 26%, total SDP increased by 9.4%, respectively. The database of apheresis platelet donor has been markedly growing. Additionally, the number of followers of the WeChat platform was continuously increasing, which deepened public's understanding and awareness of voluntary blood component donation. 36 articles illustrated with pictures and texts about apheresis platelet donation, both created and shared, were posted on this WeChat platform by the end of November 2016. The articles were read by 8,942 people, with total views of 14,927. The average reading rate hit 53%.
Summary/Conclusions: Group management of blood donors could be achieved with "WeChat + Voluntary Blood Donation" model which was a strong independent and interactive platform through which it was possible to communicate with blood donors from multi-angles. In addition, our WeChat Public Account will have more functions in the future, such as making an appointment for blood donation by WeChat and membership management, to increase the apheresis platelet donation repeatedly. "WeChat + Voluntary Blood Donation" model is worth in the practical application of promotion.

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THE SAFEST DONORS: CAN THEY BE CATEGORIZED

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Background: The safety and availability of blood and blood products for transfusion requires the recruitment and selection of voluntary non-remunerated blood donors and the quality-assured screening of all donated blood. The Dubai Blood Donation Center (DBDC) is the major blood bank of the UAE that is committed to providing a safe and adequate supply of blood. All donors in DBDC are voluntary

non-remunerated blood donors who donates either individually or as part of the company campaigns where they work or their association with nonprofit organization who organizes blood donation campaign in collaboration with DBDC.
Aims: The aim of the study is to categorize all the donors who presented for blood donation from January 1, 2016 to December 31, 2016 into 6 categories based on the business organization that they belong. And to find out the rate of pre and post donation deferrals from each category and to see if there is any association between person's usual or principal work or business and deferral.
Methods: Around 1,000 organizations both business and nonprofit are registered with DBDC for conducting blood donation campaign throughout the year. For this study, all the organizations associated with DBDC has been classified into 5 categories. These are – Service business, Manufacturing business, Merchandising business, Educational organizations and Non-profit organizations. This study includes a sixth category of donors, "the walk in donors" who does not belong to any organization and simply walks in to the Center or mobile bus for donation. The assessment of donor suitability is in accordance with AABB standards and is consistently applied in every blood donation setting on each occasion of donation to all blood donors.
Results: The pre donation donor deferral is highest from educational organization leading with 26% (1,279/3,100), followed by the merchandizing and walk ins with 18%, then service business at 16.9%. The least deferrals are from manufacturing and nonprofit organizations both at 15%. The most common cause of deferral among all the groups was low hemoglobin and across all age group except the educational organization, where low hemoglobin was mostly amongst young females (18–21 years of age). In the post donation (after lab screening) deferral, the rate of deferral varied from 14.5% to 23% amongst all the organizational groups. The least deferral was observed amongst walk in donors at 2.6% which was significantly less than all other groups.
Summary/Conclusions: The high deferral of donors amongst the student group for low hemoglobin calls for educational sessions to be conducted early in life for a healthy diet and life style. The very low post donation deferral amongst walk in donors suggests that, they are the safest donors as they come forward to donate at their own free will devoid of any peer pressure. They would defer themselves if they are aware of having been exposed to any risk of an infection or a known health condition or treatment that could influence their suitability to donate blood.

P-103

A STUDY ON KNOWLEDGE, ATTITUDE AND PRACTICE OF BLOOD DONATION AMONG UNDERGRADUATE STUDENTS IN THE UNIVERSITY OF PERADENIYA

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Background: Blood donation is a self-directed volunteer service. Younger people are the future source of blood and they are aware and have knowledge about donation but there is lack of regular donation practice among youngster. Regular donors in younger age group are less so it is very important to sensitize these people for regular donation.
Aims: To determine the Knowledge, Attitude and Practice (KAP) of blood donation among undergraduate students in University of Peradeniya. And To compare the knowledge of the blood donation between health related and non-health related categories.
Methods: A survey based, descriptive cross sectional study was conducted among 375 undergraduate students in University of Peradeniya which was selected using stratified random sampling. Data collection tool was a Self-administered questionnaire.
Results: 32.28% of the non-health related students were aware of the appropriate criteria for blood donation. Health related students had higher knowledge about the blood donation criteria (84.44%). In health related group 66.6% replied HIV/ AIDS can be transmitted to donor while donating blood and while looking at practice only 15.5% donate blood. Similarly in case of non-health group 78.59% replied HIV/ AIDS can be transmitted to donor while donating blood and 22.1% have donated blood. The reasons for not donating blood are fear of needle, transmission of infections, they think that after donation people becomes weak and their weight decreases.
Summary/Conclusions: Though the respondent has good knowledge but there is poor practice and it revealed the fact that adequate knowledge only cannot result in regular blood donation practice. The misconceptions regarding blood donation needs education and motivation through dissemination of information regarding blood donation particularly on electronic media.

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Abstract has been withdrawn.

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Abstract has been withdrawn.

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SOCIO – DEMOGRAPHIC STRUCTURE OF BLOOD DONORS IN THE REGIONAL CENTER TETOVO

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Background: Through the analysis of the socio – demographic structure of blood donors in the Regional Center Tetovo, we can achieve significant information about several crucial issues, such as plans for boosting the number of voluntary donors, as well as, programs for gaining new blood donors.
Aims: To prepare a plan for stimulating blood donation in the region, especially the young population, with an emphasis on high school students and university students, due to the fact that the population is aging.
Methods: The study covers a one – year period, starting from January 2016 to December 2016, in the Regional Center Tetovo, which includes the blood donation at the Department of Transfusiology, as well as the mobile team on field, outside the Department. The input material used for this study was data from local blood donor registry.
Results: During the analyzed period were collected 1,650 blood units. 852 (51.64%) blood units were collected at the Department of Transfusiology, while 798 units (48.36%) were collected on the field, by the mobile team. The majority of the blood units were voluntary, more precisely 1,645 units (99.70%). The rest of them were donated for family members, 5 units (0.30%). Male donors have dominant participation in the blood units, 1,455 (88.18%), while female donors 195 units (11.82%). Blood donors who have donated blood for the first time have participation of 419 units (25.39%), while blood donors who have donated blood for several times have greater participation in the blood units, 1,231 (74.61%). Employed blood donors have dominant participation of 1,038 units (62.91%), over unemployed blood donors with 122 units (7.39%). 317 units (19.21%) were collected by high school students, while 166 (10.06%) were collected by university students. Retired population participates with only 7 units (0.42%). 708 units (42.91%) were donated by Macedonians, 930 units (56.36%) by Albanians, and 12 units (0.73%) were donated by other nationalities. Even though, the percentage of blood units donated by Albanians is higher than Macedonians and other nationalities, we must put an emphasis on the fact that Albanian population is approximately 80% in Tetovo. Therefore, their participation is insignificantly higher.
Summary/Conclusions: Blood units in the Regional Center of Tetovo have been collected by two sources, which have almost equal importance. However, the study concludes that units collected at the Department of Transfusiology prevailed. Analyzed units show that the vast majority of the blood collected is voluntary. Most of the units are donated by male population. The majority of blood donors are those who have donated blood for several times. Employed population is crucial for blood donation. Our future intentions are to implement promotion activities in order to increase blood donation by females, high school and university students, unemployed people, all nationalities, especially Albanians because they are majority of the population in Tetovo.

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Abstract has been withdrawn.

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A REVIEW OF PRE-DONATION BLOOD DONOR TEMPORARY DEFERRALS AFTER THE IMPLEMENTATION OF NEW SOFTWARE AT THE INSTITUTE OF TRANSFUSION MEDICINE IN SKOPJE

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Background: The pattern of donor deferral reasons is an important blood safety issue. It provides a key area of focus when formulating a national donor selection policy, ensuring higher donor and patient safety regarding the clinical use of blood and blood components. E-Delphyn software covers all processes in a blood bank, from blood donor registration, donation, testing, production of blood components, to their storage and distribution; it is a very helpful tool, allowing physicians to easily follow each donor from his/her registration, trough produced blood components, to deferral reasons for every blood donation. In addition, the data from the software enables to create a data base and to follow up the temporary deferred blood donors and their retention in blood donor pool.
Aims: To analyze the rejection pattern of the deferred blood donors, with focus on the reasons for temporary deferral, after the implementation of a web based software- E-Delphyn.
Methods: A retrospective study was performed to analyze the number of registered, accepted and deferred blood donors, as well as the reasons for deferral, for the period from 13th of April to 31st of December, 2016. These are the first months the new software was in regular use, replacing the paper based evidence. The criteria for blood donor deferral applied in the software are based on the national blood donor regulations and recommendations from the European Directives for blood safety. Each criteria has a specific code in the software.
Results: From the total number of registered blood donors (22.674), 1,712 (7.55%) were deferred, out of which 99 (5.8%) were permanently deferred and 1,613 (94.2%) were temporary deferred. The highest number of total deferrals were registered in May (248 or 14.5%). The main reasons for temporary deferral are: low hemoglobin level in women (477 or 27.5%), low hemoglobin level in man (325 or 18.7%), "other" (193 or 11.1%), which is commonly used reason for temporary deferrals, hypertension (120 or 6.91%), hypotension (106 or 6.1%), infection (68 or 3.91%) etc. The number of permanent deferrals is decreasing from May to December (from 16.2% to 3%), while the number of temporary deferrals is steady (10.4–12.2%). According to sex, more deferrals are registered in men (55.49%) vs women (44.51%), which is in accordance with the highly predominance rate of male vs female blood donors.
Summary/Conclusions: The electronic evidence showed that the physicians included in blood donor selection, often choose the option "other" as the reason for blood donor deferral. This indicates the need for additional education, regular meetings and discussions about raising their awareness toward proper donor selection. The new software system enables easier classification of the specifically coded deferral reasons. Furthermore, it provides important data, on individual or group basis, about the blood donor history and frequency of donation. In this data way, the temporary deferred blood donors can be included in donation counseling and then re-invited for donation.

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ABSENCE OF SWIRLING IN APHERESIS PLATELET CONCENTRATES RELATED TO DONOR DIETARY HABIT: A CASE REPORT

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Background: Functional properties of Platelet Concentrates (PC) can be evaluated by swirling assessment. Up to 30% of platelet transfusions fail to achieve a satisfactory patient response 20–30% of which are estimated to be related to donor. In addition, Pathogens Inactivation (PI) has been suspected to increase the stress of platelets during processing. Swirling measurement can be performed on all PC as a pre-distribution check, as commonly carried out in some countries. The observation of platelet swirling is a non-invasive and simple method and subjective test for the non spherical shape of platelets in concentrates.
Aims: Swirling test is performed as routine check at each step of platelet processing and pre-distribution. We evaluated the frequency of absence of swirling (AOS) and tried to identify the causes of these incidents.
Methods: Apheresis Platelets (AP) are collected with Trima Accel® (Terumo BCT) or Amicus® (Fresenius Kabi) cell separator, suspended in 62% of Platelet Additive Solution -E and treated with INTERCEPT™ Blood System (Cerus) for PI. PC stored at 22 ± 2°C for 5 days are monitored daily for presence of swirling. A small number of PC was discarded for AOS as a marker for loss of functional properties. We systematically investigated AOS AP for different biochemical parameters and donor's information to identify critical steps leading to loss of functional properties evidenced by AOS.
Results: On a total of 11,223 AP collected in 2016, 92 AP were discarded for AOS representing 0.9% of the AP collected from 78 different donors.
A retrospective study on AOS incidents has identified 4 regular AP donors associated with 3 or more incidents. A regular cytapheresis female donor caught more specifically our attention. She has given 46 AP donations since July 2012 up to now. In July 2015 the first AOS incident associated with that donor was observed on 4 days storage AP. Up to September 2016, 4 AOS were observed at the same day of storage on 8 AP collected in this period of time. All other factors influencing viability of AP being excluded, a detailed interview to check habits modification of this donor has been conducted. This showed that she started to use spirulin as dietary complement just a year ago when the first AOS with her AP showed up. Spirulin with its content of phytocyane being known to have platelet activation properties it has been proposed to the donor to stop spirulin use. From this date on no new AOS have been observed on her 6 next AP collected.
Summary/Conclusions: In order to confirm a potential link between specific donors dietary habits and AOS on AP collected we developed a specific questionnaire. This questionnaire will be used in a further study focussing first on AP donors with one or several AOS incidents before extending its use to all AP donors.

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THE CZECH NATIONAL MARROW DONOR REGISTRY

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Background: In the Czech Republic are two registers donors of bone marrow. The first one is Czech Registry of Donors of Hematopoietic Stem Cells – it was founded in Institute of Clinical and Experimental Medicine (ICEM) in Prague in 1991. The second one is Czech National Marrow Donor Registry – established in Department of Hematology and Oncology of University Hospital in Pilsen in 1992 by Vladimir Koza, M.D. Currently is composited 10 Donor Centers and 41 Centers for donor recruitment. The Central Military Hospital-University is Donor Center for Czech National Bone Marrow Donor Registry for Prague and Central Bohemia region. Currently both registers counted about 95,949 donors (25.931 donors is in Czech Registry Donors of Hematopoietic Stem Cells and 70,018 donors is in Czech National Marrow Donor Registry). In total it is 0.91% of Czech population.
Aims: Statistical evaluation recruitment donors of hematopoietic stem cell in Czech National Marrow Donor Registry in 2016. Statistical evaluation collected donors of peripheral blood stem cells or bone marrow for patients in Czech Republic and for patients for foreign countries.
Methods: According to statistics the Czech National Marrow Donor Registry was recruitment 7,865 donors (3,584 male, 4,281 female) in 2016. Twelve donors (8 male, 4 female), entered to register on his eighteenth birthday. Average age was 25 years.
Results: Czech National Marrow Donor Registry collects unrelated donors for all transplant centres in Czech Republic. The registry searches compatible donors for patients in the Czech Republic also for foreign patients. In 2016 was collected 44 donors of this registry (9 BM, 35 PBSC). The bone morrow was removed for 4 patients from Czech Republic and for 5 foreign patients (states: DEU, CHE, POL, USA). The peripheral blood stem cells were collected for 24 our patients and for 11 foreign patients (states: DEU, HUN, ISR, ITA, NLD, USA). The Czech National Marrow Donor Registry provide import of stem cells for our patients too (n = 64).
Summary/Conclusions: Czech National Marrow Donors Registry provide 2/3 unrelated transplantations in Czech Republic. From the total amount of successful transplants in Czech republic is 20% from Czech national bone marrow donor registry. In comparison with other developed countries, it is the extraordinary result, because for example Switzerland and the Netherlands have only 3%, or 4% donors from their national registry. The collections are performed in internationally accredited Collection center at University Hospital in Pilsen. For typing of patients and donors serves the central HLA laboratory accredited by EFI. In March 2005 the Czech National Marrow Donor Registry underwent the accreditation WMDA that in the years 2010

and 2014 successfully vindicated. The most successful year to recruit new donors was 2015, when entered into the register 9,759 new donors. For success is excellent cooperation registry with Donor Centers and Centers for donor recruitment. Also great thanks for the media coverage of this program.

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THE IMPORTANCE OF CARDIAC SCREENING IN YOUNG BLOOD DONORS

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Background: In order to protect the health of the blood donor, at the AVIS collection center was implemented cardiac screening program exclusively for young donors.
Aims: The aim of this study was to make as survey of first level: electrocardiogram (ECG) to detect alterations of cardiac function (arrhythmias, myocardiopathies, long QT syndrome, Brugada syndrome etc). This survey allows to identify silent and undiagnosed cardiac abnormalities, which could in extreme cases lead to sudden death.
Methods: The study was conducted from September 2016 to January 2017 at the AVIS collection center. All selected donors were Southern Italian and Caucasian origin, aged between 18 and 25, that have been subjected to 12-lead ECG (Esaote P8000 Power). Ten electrodes are placed on the patient's limbs and on the surface of the chest. The overall magnitude of the heart's electrical potential is then measured from 12 different angles ("leads") and is recorded over a period of time (usually 10 seconds). An ECG machine records these electrical signals across multiple heart beats and produces an ECG strip that is interpreted by a healthcare professional.
Results: In this period were conducted 600 electrocardiograms. We observed that the 2.7% of our study population showed an altered profile. Seven subjects (1.16%) displayed repolarization abnormalities; respectively four with long QT syndrome and three with Brugada syndrome. Nine (1.5%) showed a hypertrophic cardiomyopathy. These subjects with abnormal ECG were referred to second level screening (cardiology consult, echocardiogram) and if necessary to third-level investigations (electrophysiological study).
Summary/Conclusions: These results indicate that the ECG showed good sensitivity and specificity in order to identify patients with potentially lethal cardiomyopathies and/or channelopathies. We can conclude to better protect the health of the donor, is necessary to develop a cardiac screening protocol that uses the 12-lead ECG. This one, in fact represents a valuable tool that helps to assess the risk of cardiovascular events and sudden cardiac death of asymptomatic subjects.

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BLOOD DONATION STRATEGY, BASED ON PLURIDISCIPLINARY APPROACH OF REJECTION FEATURES, ACCORDING TO CRITERIA OF ADMISSION/POSTDONATION, IN BIHOR COUNTY ROMANIA, DURING 2011–2016

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Background: One of the most important approach in transfusion medicine, is the protection of both donors and recipients. The paper displays sequentially, predonation/postdonation eligibility assessment, the features of rejection criteria, of potential/blood donors, according with different valuable clinical/paraclinical aspects.
Aims: Due to the large panel of temporary and permanent deferral criteria, the evaluation of the potential/blood donor, is very important, according to demographic/geographic/gender, and pathology features.
Methods: The data were collected from the donors' files and medical records, during 2011–2016, in BTC of Bihor County, Romania. The data referred to: the total number of potential/blood donors/number of donation/blood donors' category/gender/predonation/postdonation features of deferral criteria, and the returning rate of the individuals with temporary deferral.
Results: Over the studied period, the percentage of potential blood donors from the total number of blood donations per year, was about 80–85; the ratio between total number of blood donors/ first blood donors' category, increased significantly: 20% (in 2011) to 42.3% (in 2016). The gender was mainly represented by men, women being in 2011: 28%, from the total number of blood donations, in comparison in 2016: 45%, and 34.5% in first donors' category in 2011, and 61% in 2016. The difference between the potential/blood donors, was covered by permanent/temporary deferral criteria, divided into: lower Hb level, cardio-vascular, other medical diseases, sexual /habitual risk behaviors and non-medical reasons. The main cause of temporary rejection, was represented by lower Hb (over 40% of the total number of rejected individuals) – with slight variation; the second temporary deferral criteria was represented by increased ALT (about 15% from the total number of rejected individuals) with the same trend as Hb. The panel of cardio-vascular and other medical diseases, were represented by more than 30 different diseases (mainly by HBP, cardiac arrhythmia, and diabetes mellitus). From the postdonation criteria, with temporary (repeated/predonation reevaluation) and permanent rejection criteria, the ratio between causes was maintained along the studied period, with less significant variations, as follows: ALT (about 75%) > AgHBs (about 11%) > Treponema pallidum EIA (about 7%) > Ag/Ab HCV (about 3.2%) > Ag/Ab HIV ½ (from 0% in 2011 to 2.3% in 2016) > Ac HTLV I/II (about 0.2%). The return rate of the temporary deferral potential/blood donors, was similar in both, about 40%.
Summary/Conclusions: The reported data, show the importance of medical consult and laboratory tests results, based on high professional staff, of potential/blood donors, having as major aim, transfusion safety. The better strategy in selection procedure, was noticed along the studied period, with obvious positive results in the biological quality of the blood donors' pool, the return rate being supported by a good collaboration with physicians of different specialities, and in case of permanent deferral, a correct diagnosis in due time, with proper treatment and higher quality of life, shows the importance of blood centers activity, as source of valuable and helpful medical information.

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THE MOST FREQUENT REASONS OF DONORS DEFERRAL AT THE DEPARTMENT OF TRANSFUSION MEDICINE IN STRUMICA

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Background: Blood centres have a primary obligation to protect the health of donors. Therefore all donors must undergo a screening process to assess their suitability. Only healthy persons with a good medical history can be accepted as donors of blood or blood components.
Aims: The aim of this paper is to demonstrate that in the process of selecting donors who can donate blood without any damage to their health, a careful medical examination, a proper questionnaire and a correct evaluation of every potential donor are the basic steps in taking care of donors' health.
Methods: The process of selecting donors who can donate blood without any damage to their health was carried out on 2079 potential donors who came to donate blood at the Transfusion Department - Strumica in 2016. The screening of donors was conducted through interviews, a questionnaire, direct questions, measuring of Hb levels, measuring of blood pressure and auscultation of heart.
Results: In 2016, 2079 individuals volunteered to donate blood in the Department of transfusion medicine in Strumica, 1902 (91.5%) of whom donated blood while 177 (9.3%) were deferred. The reasons for deferral are the following: low Hb in women (below 125 g/l) – 47 (26.5%), low Hb in men (below 135 g/l) – 53 (29.9%), infections treated with antibiotics – 6 (3.4%), hypertension – 24 (13.5%), hypotension – 2 (1.1%), tattoo in the last 12 months – 3 (1.7%), lower body weight (lower than 50 kg in women) – 9 (5.0%), a period less than three months since the last blood donation in men – 3 (1.7%), a period less than four months since the last blood donation in women – 3 (1.7%), surgeries in the last 12 months – 4 (2.2%), taking medicines – 4 (2.2%), diseases (diabetes mellitus – insulin dependent, heart diseases, allergies) – 4 (2.2%), other – 15 (8.4%). All of these potential donors were deferred for a certain period of time after which they could volunteer to donate blood again.
Summary/Conclusions: The questionnaire must be designed to elicit information relevant to the health and life style of the donors. The person who carries out the assessment has to make a decision about the donor's health on the basis of appropriate questions and a careful medical examination. Well-conducted screening leads to protection of the donor's health as the first priority of blood donor management. Deferred donors must be given a clear explanation of the reasons for deferral.

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FIRST BLOOD DONATION, RECRUITMENT AND RETENTION OF DONORS

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Background: In our country citizens become eligible for blood donation at the age of 18. Therefore the first blood donation is usually associated with high school students. This population also represents the most important source of new donors who donate blood for the first time because they are young and healthy on the one hand and on the other hand they are enthusiastic and always willing to try something new and to help someone.
Aims: It is very important to undertake numerous activities aimed at recruiting the largest possible number of new young blood donors as well as activities for their retention as regular blood donors.
Methods: The Institute for Transfusion Medicine of the Republic of Macedonia in cooperation with the Red Cross of the Republic Macedonia implement voluntary blood donation campaigns in secondary schools in the whole country. The preparation of the campaigns implies timely and accurate information about the time and venue of the blood donation campaign, informative and educational lectures about the importance of voluntary, anonymous, unpaid and regular blood donation, information on the importance of ensuring sufficient supplies of blood and blood components, the importance of self-exclusion from the blood donation process by correct and accurate completion of the questionnaire that donors must fill in before each donation, suggestions on how to behave during and after blood donation as well as conducting surveys in secondary schools in order to improve our work with young blood donors.
Results: During 2016 a total of 1902 blood donors donated blood at the Department of Transfusion Medicine - Strumica, the Institute for Transfusion Medicine of the Republic of Macedonia. There were eight blood donation campaigns in four secondary schools. During these blood donation campaigns a total of 220 (11.56%) students donated blood out of whom 139 (7.30%) students were first-time donors, and 81 (4.25%) student were second-time donors. These figures demonstrate a large response among young people who donated blood for the first time, and at the same time a large number of students who continued to donate blood.
Summary/Conclusions: Well organized blood donation campaigns and implementation of the already mentioned activities as well as motivation and raising awareness of the importance of regular voluntary blood donation contribute to a greater response by students for first blood donation.

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Abstract has been withdrawn.

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Abstract has been withdrawn.

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BLOOD DONATION: GENERAL PERCEPTION OF UNIVERSITY STUDENTS IN LAHORE, PAKISTAN

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Background: Blood transfusions are an integral part of the medical management of a number of diseases. The shortage of blood in Pakistan is owing to an increase in the demand, with limited voluntary donations. The collection of blood from voluntary non-remunerated blood donors (VNRBD) is an important measure to ensure availability and safety of blood.
Aims: The current study was conducted to find out the factors which motivate our youngster to donate blood and to know the barriers which impede them for blood donation and to comprehend the situation and find ways to enhance voluntary blood donation.
Methods: In this cross section qualitative study, a detailed questionnaire was designed, comprising of questions about their previous donations, experiences, and reasons for donating or not donating. Data were analyzed by IBM SPSS version 21.0.
Results: Out of the 789 participants, 51.46% (n = 406) were males and 48.54% (n = 383) were females, with mean age of 21.65 ± 2.98. Only 36.0% (n = 284) were blood donors including 81.39% (n = 231) males and 18.61% (n = 53) females. Helping others, altruism, sense of social responsibility, volunteerism, spiritual pleasure and gaining experience were major factors for blood donation while never asked for donation, objection from elders, fear for the needle, fear of adverse effects, weight gain/loss were the barriers which impede our youngsters for blood donation.
Summary/Conclusions: To increase the voluntary blood donations, the younger population must be counseled so that all the myths and the false beliefs regarding blood donation can be mitigated.

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BEHAVIOR AND MOTIVATIONS OF BLOOD DONORS AT BLOOD AND TRANSFUSION MEDICINE DEPARTMENT (SSMT), COIMBRA HOSPITAL AND UNIVERSITY CENTER (CHUC)

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Background: Motivation of blood donors.
Aims: Understand the donor behavior and motivations of SSMT-CHUC donors.
Methods: Between January and February 2017, 1604 donors were surveyed (12,325 donors in 2016, 99% confidence interval, 3% sampling error) about number, periodicity, planning of the donation and motivational variables adapted to the donation of blood and adjusted to the population and context under study. Data were analyzed using Excel® and SPSS®.
Results: Most of the donors are me (52%), are between 25 and 44 years old (47%), mostly with secondary education (34%), living in the district of Aveiro (36%) and Coimbra (36%). 66% donations were collected at a mobile site (CSLM), mainly distributed in the districts of Coimbra (48%), Aveiro (35%) and Leiria (13%) and 34% at CHUC. 5% are first time donors, 1% have made 1 donation; 32% ≥2 donations and 58% are regular donors (≥2 donations in the last 2 years in SSMT-SHUC). The main motivations of the 1st donation (≥1 motivations): "helping others" (84.4%), "reciprocity" (46.5%), "community help" (4%). Present motivations: "helping others" (66.4%), "reciprocity" (37%), "habit" (31.8%), (16.3%). Deterrents of donation initiation (≥1 options): (28.2%), "not knowing local/timetables" (23.5%), "lack of knowledge" (48.2%), "never thought about it" Medical reasons (21.1%), "fear of needles/blood" (18.8%). Frequency of donation: 28.7% makes the donation with the allowed regularity, with the most significant deterrents being "inconvenience" (23.3%), "forgetfulness" (13.2%) and "medical issues" (12, 1%). Analyzing the context, 57% refer to planning the donation, 26% said to have been summoned and 17% mentioned not having been planned and simply having provided. Comparing the CSML and CHUC donor profile, the main differences are: greater% of the "25–44" age group in the CSLM and greater% of the "18–24" age group in the CHUC; In both, the secondary level is the most frequent followed by the 3rd cycle in CSLM (22.0%) and Bachelor's degree (25%) in CHUC. Most CSLM donors reside in the districts of Aveiro (52.7%) and Coimbra (74.1%). The motivations of the 1st and present donation are similar, being the main difference:"geographical proximity" in the current CSLM donation. The main deterrents for 1st CSLM donation are: "inconvenience" (31.8%), "never thought about it" (23.5%), "fear of being ill" (20%). In CHUC: "I never thought about it" (18.8%), "inconvenience" (16.5%), "medical issues" (11.8%). The most frequent deterrents are "inconvenience" (13.6% -CSLM, 9.7% -CHUC), "medical issues" (8.4% -CSLM, 3.7% -CHUC) and "forgetfulness" (7, 0% -CSLM, 6.3% -CHUC). In spite of the majority having a planned donation (57.8% -CSLM, 59.4% -CHUC), in CSLM 35.6% reported having been summoned (only 7.3% in CHUC), with CHUC having a larger unplanned donation (30.7% -CHUC, 10.10% -CSLM). Comparing the context (≥1 response options) with donor frequency, regardless of the donor career step, most plan the donation. Of those summoned, the majority (27.6%) is regular SSMT-CHUC donors; Of those who didn't plan the donation, the majority (31.6%) are first time donors.

Summary/Conclusions: It was possible to trace the SSMT-CHUC donor profile, with the main motivations being: pro-social, reciprocity and convenience, being the "habit" relevant to the current donation. Differences in the profile of the donor may be subject of intervention to motivate and retain donors.

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DEMOGRAPHIC TREND OF FIRST- TIME BLOOD DONORS

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Background: Due to changes in demographic pattern of the Iran population and for meeting patients need,encouraging first-time donors to give blood and make it a lifelong habit in order to meet the patients needs is necessary. So, monitoring the demographic profile of first-time donors and evaluating changes in the first-time donor pool is necessary.
Aims: The aim of this study was to analyze demographic factors among first time blood donors in Shiraz donation data are needed.
Methods: This cross-sectional study was conducted at Blood transfusion center in Shiraz, Iran from 2012 to 2016. Demographic factors (job, marital status, and gender and education status) of donors were evaluated.
Results: The total number of first-time during study period were 101,281. The results indicate decreased the percentage of first time donors from 19.7% (2012) to 15.5% (2015).However, the demographics of (age, gender, education, and job) of donors did not change during study period. In this study, about 67.9% of first-time donors had low education, 69.7% low profession job, 3.8% female, and 27.5% were single.
Summary/Conclusions: The number of first-time donors is decreasing over the time. The demographic profile of first-time donors did not change. These data highlight the importance for blood centers to recruit women, high educated individuals for blood donation. A better understanding of the donor population may help blood centers to design recruitment programs.

P-120

Abstract has been withdrawn.

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KNOWLEDGE, ATTITUDE, AND PRACTICE OF ADULT POPULATION TOWARDS BLOOD DONATION IN GONDAR TOWN, NORTHWEST ETHIOPIA: A COMMUNITY BASED CROSS-SECTIONAL STUDY

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Background: Though World Health Organization recommends 100% voluntary blood donation, the percentage of blood collected from voluntary blood donors and the average annual blood collection rate are extremely low in Ethiopia. The role of adults is crucial to meet the demand of safe blood.
Aims: To assess knowledge, attitude, and practice of adult population towards blood donation in Gondar town, Northwest Ethiopia.
Methods: A community based cross-sectional study was conducted among 768 adults. Multistage sampling technique together with simple random and systematic random sampling technique was employed. Bivariate and multivariate logistic regression analysis and bivariate correlation analysis were done.
Results: About 436 (56.8%), 630 (82%), and 141 (18.4%) study participants had adequate knowledge, good attitude, and experience of blood donation, respectively. Secondary and higher educational statuses were significantly associated with adequate knowledge towards blood donation. Participants who were protestant by religion were more likely to have good attitude towards blood donation. Age, self-perceived health status, and religion were significantly associated with blood donation practice.
Summary/Conclusions: Knowledge and attitude towards blood donation are high. However, the level of practice is low. District and national blood banks and transfusion agency should design strategies that promote and motivate the communities to donate blood.

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MOTIVATIONAL MEANS FOR BLOOD DONATION IN THE REPUBLIC OF MACEDONIA

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Background: Blood donation in the Republic of Macedonia is based on the general, global established principles: Willingness, Anonymity, and Voluntary, without discrimination in relation to the social status, race, nation, religion, class or political orientation. The following two days given as days off after the blood donation, the small gifts, the refreshment after the donation, the control of the health condition, are compatible with the basic postulate for voluntary blood donation without compensation.
Aims: To show the reasons for blood donation in the last period of five years in the Department for Transfusion Medicine in Gevgelia.
Material and methods: A period of five years is analyzed, from 2011 to 2016. There was a survey among the volunteers from all age groups, men and women from 18 to 65 years old that have donated blood in the Department for Transfusion Medicine, Gevgelia.
Results: From the total number of 2,629 voluntary blood donators, 1,720 (65.4%) were employees, 698 (26.6%) voluntary blood donators were unemployed, 182 were students (6.9%), and the rest were 29 (1.1%) VBD. 1,902 voluntary blood donators (employees and students) were included in the survey. From them, 1,100 (57.8%) explained that they come to donate blood due to altruistically inspiration, because this fulfils them, by giving benefits to the others and rescuing the life of other people. A total number of 640 (33.6%) come to donate blood due to the two days off that are given to them after the blood donation, and the rest 162 (8.5%) donate blood because they feel better after the blood donation.
Summary/Conclusions: In the last years, the voluntary blood donation is in increase and satisfies the needs for blood in our institution. The current motivational factors, like the following two days off after the blood donation, which come from the present existing Laws in the Country, still have great influence on the employees and the students as voluntary blood donators. The created long standing tradition for blood donation in our Municipality, the devotion of the personnel and their flexibility in the work and the volunteers that care about the animation of new blood donators have a key role in the motivation of the blood donators, too. They are key factors for securing and collecting voluntary amounts secure blood from own origin.

P-123

Abstract has been withdrawn.

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THE BLOOD DONORS DEFERRAL RATE IN AHVAZ BLOOD TRANSFUSION SERVICE (3 YEARS EXPERIENCES)

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Background: The safety of blood donors and recipients are the cornerstone of all blood transfusion services. The improper deferral rate threatened the blood supply or patients or blood donors lives.
Aims: Due to high prevalence of blood dependent patients in Ahvaz, evaluating the deferral rate help us the blood supply safety and to get preventive or corrective actions.
Methods: The study was retrospective cross-sectional and included all blood donors that differed permanently or temporarily during 2013 to 2015 in Ahwaz blood transfusion service. The data analyzed by using SPSS16.
Results: We found that the numbers of admitted people for blood donation reduced from 157,602 people in 2013 to 148,390 in 2016. The deferral rate reduced from 27% to 22% at that period and the viral marker were decreased, but in last year showed slightly increase. Around 73% of deferred people was diploma or less certificate. 93% of deferred donors was men.
Summary/Conclusions: According to our findings the rate of differed reduced and the viral markers rate decrease in that period that showed effectiveness of donor selection. It's so important to be aware about decrease donor deferral rate, as we found higher viral marker when we reduced the deferral rate. The differed donors should be well educated to increase their knowledge. The blood donor recruitment should be more active to increase the admitted blood donors.

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Abstract has been withdrawn.

Blood collection incl. apheresis

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CAN "HISTORICAL DONOR PLATELET COUNTS" RESULTS BE USED FOR PROGRAMMING PLATELET DONATION?

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Background: Platelet count and haematocrit values are used for programming apheresis platelet collection. Standard practice was to test a FBC in the collection centre prior to donation and use these results to program platelet collection. Using historical platelet counts instead is accepted by the manufacturer and this approach was strongly supported by local data from occasions when in-centre analysers were out of action. Using a historical count reduces costs and simplifies the logistics of platelet donation.
The Australian Blood Service implemented a process change; Act2Diff analysers were removed from donation centres. FBCs are tested centrally and apheresis platelet collection is now based on historical platelet counts.
Aims: This study was to confirm that apheresis platelet yields would remain within specification while using historical platelet counts and also confirm that the historical values would reliably predict current donor platelet count.
Methods: Routine Quality control data from platelet components tested from a 6 month period prior to implementation (1st October 2014- 31st March 2015) was compared with results for 6 months after implementation (1st October 2015- 31st March 2016). Mean historical platelet counts used for programming platelet collection were also compared with the platelet count obtained on the day of donation. The historical platelet count was obtained from an average of up to 3 results within 2 years. A target was set for 90% of samples to have less than 20% variance between the historical platelet count and the value obtained on the day of collection based on previous data this would ensure platelet yields remain within specification.
The change was implemented in a nationally staged approach. Automated platelet collection includes the use of predicted platelet yield, calibrated by yield scaling and adjustments were performed prior to and immediately following the changeover at every site. An initial increase in yield target, up to 3.2%, was included to provide confidence that any fluctuations in platelet yield would not compromise maintenance of platelet specification. A broader review in order to maintain donor safety was also undertaken in conjunction with this change.
Results: Pre-implementation, 423 single donations and 2216 double collections were analysed and post- implementation 573 single and 2116 double collections. The component yield (platelet count $\times$ 10^9 per unit) pre-implementation was 273.3 $\pm$ 32.0 (n = 2639) and post-implementation was 282.8 $\pm$ 38.8 (n = 2689) (Independent *t*-test $P < 0.001$). Over 95% of platelet count results obtained on the day of donation fell within 20% of the historical estimate with all counts remaining $> 140 \times 10^9$/l.
Summary/Conclusions: The removal of ActDiff analysers from donor centres and replacement with the use of historical mean platelet counts was successful. The use of historical platelet counts does not result in a reduction in donor safety or component quality. Removal of the Act2Diff analysers has reduced in-donor centre workload, simplified regulatory compliance and markedly reduced the cost associated with platelet collection. It has also eliminated the work health and safety issues and risks associated with donor staff operating full blood count analysers.

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FIFTY YEARS OF RHD IMMUNOGLOBULIN THERAPY IN AUSTRALIA

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Background: At the 11th Congress of the ISBT in Sydney in 1966, researchers from Liverpool announced the first successful trials of Rh D immunoglobulin (Ig) derived from human plasma to prevent the effects of Rh D blood group incompatibility between a mother and her baby. Soon after, Australia's state-based Red Cross Blood Transfusion Services began identifying Rh D negative women with strong reactions to their Rh D positive babies, and whose Rh D antibodies could be used to treat other women prophylactically. It soon became apparent that, in addition, immunised men could provide a good donor base to provide plasma for this program. Australia's Rh D immunisation program began in August 1967 and celebrates its 50th anniversary this year.
Aims: We review the outcomes of Australia's Rh D immunisation program in reducing haemolytic disease of the newborn. The ability to maintain self-sufficiency of supply of Rh D Ig for Australia is examined. Past and future scientific developments are outlined.
Methods: Plasma collection began from women immunised during pregnancy and males with pre-existing anti-D following transfusion, and subsequently from men and women (of non-childbearing potential) who were immunised with small volumes of Rh D positive red blood cells. Rh D Ig was produced by Cohn fractionation. In 1969, Australia became the first country to be self-sufficient in Rh D Ig, which was used postnatally to treat all Rh D negative women with Rh D positive babies. Australia began experiencing shortages of domestically produced Rh D Ig in 1995, and began importing product to supplement local supply. In 1999, guidelines recognised that routine antenatal prophylaxis was regarded as best practice, but it could not be recommended at the time due to supply constraints. A staged implementation of routine antenatal prophylaxis with Rh D Ig given to all Rh D negative pregnant women with no preformed anti-D at 28 and 34 weeks' gestation was subsequently made possible through a number of measures, including expansion of the anti D plasma collection program and introduction of a 250 IU dose of RhD Ig for sensitising events in the first trimester. Full implementation of routine antenatal prophylaxis was achieved in 2006 when Australia once again became self-sufficient in Rh D Ig.
Results: Despite continuing through the HIV and hepatitis C epidemics of the 1980s and 1990s, there have been no reports of viral transmissions from Australian Rh D Ig. A hallmark of the success of the project has been the dedication of a panel of around 150 donors in any one year to provide plasma for the program. In particular, one donor has donated to the program since its inception 50 years ago and has made almost 1,200 donations in total. It is estimated that his plasma has contributed to every batch of Rh D Ig made in Australia.
Summary/Conclusions: Australia's Rh D immunisation program is a great tribute to the donors who have provided red cells for Rh D hyperimmunisation, and the donors who have consented to be immunised and/or boosted with these red cells in order to provide plasma for the program over the 5 decades of its history. Haemolytic disease of the newborn is now a rare disease in Australia.

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HIGH-DOSE INTRAVENOUS IRON VERSUS ORAL IRON IN BLOOD DONORS WITH IRON DEFICIENCY: THE IRONWOMAN RANDOMISED, CONTROLLED TRIAL

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Background: Iron deficiency is very common in blood donors, but for various reasons, it is currently rarely assessed or treated. Modern intravenous (IV) iron preparations are well tolerated and allow for application of single doses up to 1 g.
Aims: Our aim was to compare a standard oral iron preparation to the application of a single large dose of intravenous ferric carboxymaltose. Our hypothesis was that IV iron is more efficient and as safe as oral iron supplementation.
Methods: In the IronWoMan randomised controlled trial we included 38 male and 138 female blood donors (87.4% whole blood donors and 12.6% platelet apheresis donors) aged $\geq$18 and $\leq$65 years with iron deficiency without anaemia at the time of blood donation (n = 176, ferritin $\leq$30 ng/ml). Stratified by gender, at visit 0 (V0, 4 to 8 weeks after blood donation), participants were randomized with a web-based randomisation tool in a 1:1 ratio to either 1 g of intravenous ferric carboxymaltose (IV, n = 86) or 10 g of oral iron fumarate (PO, n = 90, 100 tablets of 100 mg). Participants were assessed after 8 to 12 weeks at visit 1 (V1). 4 participants (4x PO, 3 female and 1 male donor) were lost to follow-up. The primary outcome of the study was the difference of transferrin saturation at V1 between the two study groups (IV versus PO).

Results: Transferrin saturation levels after iron therapy were statistically significant higher in the IV group compared to the PO group (mean ± SD: IV 29 ± 10%; PO 24 ± 13%; P 0.001) Ferritin levels increased significantly in both groups, but more in the IV group (mean ± SD: IV 7 ± 6 to 111 ± 59 ng/ml; PO 11 ± 16 to 28 ± 21 ng/ml; P < 0.001), while soluble transferrin receptor decreased significantly in both groups, respectively more in the IV group (mean ± SD: IV 2.11 ± 0.66 to 1.07 ± 0.23 mg/l; PO: 2.21 ± 0.6 to 1.35 ± 0.28 mg/l, P < 0.001). Haemoglobin levels were identical at V1 (mean ± SD: IV 13.7 ± 0.9 g/dl vs PO 13.7 ± 1.0 g/dl), but mean cellular haemoglobin and mean cellular volume increased more in the IV group. Compliance was excellent (dropout rate 2.3%, 4/176). No serious adverse events occurred in both cohorts and > 80% would recommend the therapy they received.
Summary/Conclusions: Intravenous iron was more effective in improving the iron status of iron deficient blood donors compared to oral iron, while haemoglobin levels improved similarly in both groups. Oral and IV iron were very well tolerated. In theory, IV iron allows for an elegant one-stop approach for correcting iron deficiency in blood donors, but given the high costs and lack of practicability in mobile donation settings, oral iron appears to be an acceptable alternative.

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PROVISION OF RARE BLOOD – CELEBRATING TEAMWORK AND SUCCESS

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Background: During 2016/17, NHS Blood and Transplant (NHSBT) was asked to provide blood for a 31-year old antenatal patient with a complex combination of red cell antibodies (anti-Fyb, anti-Jkb, anti-M, anti-S, anti-K). Despite this being her fifth pregnancy, the patient had delivered only one live baby. The Doppler MCA PSV at 17 weeks gestation demonstrated that the fetus was anaemic during specialist ultrasound examination. Intrauterine transfusions (IUTs) were suggested to prevent potentially fatal fetal anaemia and hydrops fetalis. We describe the teamwork required to achieve a successful outcome.
Aims: To identify and bleed compatible blood donors in order to support the pregnancy until viable for delivery. To meet the specification for IUT – use within 5 days of donation, compatible with the mother, i.e. negative for clinically significant antibodies, RhD negative, CMV negative, HEV negative, irradiated and sickle cell haemoglobin negative. The red cells are suspended in CPD-anticoagulated plasma within 12 h of venepuncture, with a target haematocrit of 0.70–0.85 l/l.
Methods: We searched NHSBT's donor database for suitable donors to invite to donate using our documented "special call-up" procedure. This procedure is initiated once or twice each month, with requests ranging from one to several units of red cells, sometimes over a period of several weeks. It is effective in supplying blood for patients with rare blood types, in the UK and abroad. Eighty suitable donors were identified to provide blood for this antenatal patient but the list was halved when accounting for the date of previous donation, medical deferrals, travel and illness. Our next challenge was to find donation sessions that were located near to the donor's home or workplace, within 5 days of the IUT but allowing time for manufacture and testing.
Two donors were called up for each IUT. Session details were confirmed in writing and donors were thanked for responding to our call. Details were provided to staff at the relevant blood collection sessions and manufacturing centres so that everyone was ready to receive and process the donations. Due to the special nature of this case, Medical staff tracked the progress of the donations at every stage.
Results: Red cells were supplied for a total of nine IUTs, at regular intervals between September and December 2016. The last IUT was performed between Christmas and New Year. We could not have supported this treatment program without the goodwill of our donors and the dedication of our staff, particularly during a busy holiday period. In January 2017 the patient delivered a healthy baby at 34 weeks gestation. The baby received an immediate red cell exchange transfusion.
Summary/Conclusions: Transfusion support for IUT is demanding and requires careful planning and coordination. This success story is the result of effective communication and excellent teamwork by the transfusion service and the Fetal Medicine Unit. It highlights the care and dedication of staff, and the wonderful generosity of our blood donors.

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BLOOD DONORS' PERCEPTION ON ABO BLOOD TYPE BASED MANAGEMENT AND ITS INFLUENCE ON INTENTION TO DONATE

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Finnish Red Cross Blood Service (FRC BS) provides blood components for the population of 5.5 million in Finland. The demand of blood is slowly declining in Finnish hospitals. The FRC BS´s aim is to efficiently adjust the donations to the need of patients.
The message to blood donors has changed. Until 2014, FRC BS advised donors to give blood regularly 2 to 3 times per year regardless of the ABO blood type. To optimize the blood supply, the concept of donor guidance was created and it is based on each donor´s individual ABO blood type since 2014.
The concept consists of the following: Nurses give guidance to each donor face to face. The donors are advised to check the blood supply barometer online before donating. Individual guidance is given to every donor according to their ABO blood type. Depending on the donor's blood type, the message is specified. The donors are given ABO specific pins and bookmarks. In addition, invitations to donate are generated according to donor´s ABO blood type and ABO inventory. The donors are instructed to donate when they are invited. The ABO guidance concept was also implemented systematically into all marketing materials in all channels.
The aim of the study is to investigate the donors' perception on ABO blood type guidance given by nurses and its influence on the donor's intention to donate.
We studied the data from two quarterly blood donor surveys from Q1/2014 (n=1011) and Q3/2016 (n=2235).
We analyzed the donors' perception of getting ABO guidance during the visit and if the information about the need of their blood type influences their donation behavior.
The number of responding donors was 2233 and 1008 in 2016 and 2014, respectively.
In the 2016 survey, 24.2% of the respondents reported that nurses discussed the meaning of their blood type, compared to 16.0% in the 2014 survey. The difference is statistically significant (OR 1.68 95%CI 1.38-2.04).
Furthermore, in 2016 60.9% of the donors felt that information on their blood type influenced their donation behavior, as compared with 46% in 2014. The difference is statistically significant (OR 2.07 95%CI 1.41-3.05).
The concept of ABO blood type guidance was implemented systematically into all marketing materials in all channels. As a part of the new concept, guidance given by nurses has been beneficial. The conceptualization of ABO blood type based donor management has been favorable in getting the intended blood type specific messages through to donors. It also seems to influence positively the donors' intention to donate according to the need of their blood type.
The change can already be seen in the red cell inventory. In 2016 the red cell inventory has improved, corresponding the target of 4-6 days 69.5% of the time (2014: 60.6%).
To keep the blood supply optimal in our organization (FRC BS), the ABO blood type based guidance is regarded vital.

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PHYSIOLOGICAL STRESS RESPONSE PATTERNS DURING A BLOOD DONATION

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Background: Donating blood has been associated with increased stress responses, and evidence suggests increased levels of psychological stress around the moment of needle insertion. Incidentally, studies have investigated physiological stress responses, such as blood pressure or heart rate, but results are contradicting.
Aims: By combining multiple physiological markers at key moments during a donation procedure, we aimed to assess whether a blood donation induces physiological stress responses in whole blood donors, and differences between men and women, first-time and experienced donors, and donors high and low on non-acute stress.

Methods: In 372 donors, physiological stress was measured during a routine donation. We assessed blood pressure, pulse rate and pulse rate variability (PRV). PRV was assessed using the root mean square of successive differences (RMSSD), high frequency (HF, 0.15–0.4 Hz) power, and low frequency (LF, 0.04–0.15 Hz) power. In general, lower levels of RMSSD, HF and LF are indicative of more stress or activity. Gender and donation experience were retrieved from the donor database. Non-acute stress was assessed by a questionnaire. To assess the shape and significance of time course patterns, multilevel models were fitted. Group-effects (main and interaction) were tested by separately adding gender, donation experience and non-acute stress. Only significant effects ($P < 0.05$) are reported.

Results: Significant time course patterns were found for all stress measures. Overall, levels of systolic blood pressure (range means 129–145 mmHg, $F(1,1315) = 24.2$, $P < 0.001$), diastolic blood pressure (range means 74–81 mmHg, $F(1,1326) = 50.9$, $P < 0.001$), RMSSD (range means 39.7–53.6 ms, $F(1,1315) = 24.2$, $P < 0.001$) and HF (range means 658–1,202 ms^2, $F(1,1624) = 34.0$, $P < 0.001$) increased toward needle insertion, dropped at needle uncoupling, and then increased to a value lower than when arriving at the donation center. Results for LF (range means 1,095–2,416 ms^2, $F(1,1627) = 14.1$, $P < 0.001$) were similar, with an additional drop at the registration desk. Pulse rate (range means 73–86 beats per minute, $F(1,1393) = 507.4$, $P < 0.001$) showed a *U*-shaped curve, with highest values when arriving and leaving the donation center. The following significant group-effects were found: women compared to men had a higher systolic blood pressure (main effect gender $F(1,335) = 3.9$, $P = 0.048$) and pulse rate (main effect gender $F(1,347) = 14.0$, $P < 0.001$); first-time compared to experienced donors had a higher pulse rate, with a decreasing difference toward the end of the donation (main effect donation experience $F(1,307) = 16.0$, $P < 0.001$, interaction effect $F(1,286) = 5.2$, $P = 0.023$); first-time compared to experienced donors had a higher RMSSD at arrival, and from the screening until leaving the donation center (interaction effect $F(1,1642) = 12.3$, $P < 0.001$); first-time donors had stable high levels of LF from registration desk until uncoupling (interaction effect $F(1,1668) = 8.9$, $P = 0.003$); first-time donors showed a decrease in HF from arrival to the registration desk, whereas experienced donors showed an increase (interaction effect $F(1,1656) = 12.2$, $P < 0.001$).

Summary/Conclusions: In conclusion, our results indicate an increase in physiological stress toward needle insertion, followed by a decrease until leaving the donation center. This response pattern is comparable for men and women, first-time and experienced donors, and donors low or high on non-acute stress.

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THE IMMEDIATE EFFECT OF BLOOD DONATION-INDUCED STRESS ON COAGULATION PARAMETERS

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Background: Blood donation has been shown to induce stress responses like increased anxiety and blood pressure. Moreover, in non-donation settings, acute stress has been reported to increase coagulation parameters, such as factor VII (FVII), factor VIII (FVIII), fibrinogen, and hemostatic factors like von Willebrand Factor (vWF), whereas overall clotting times such as prothrombin time (PT) and activated partial thromboplastin time (aPTT) remained unaltered. Although this association between stress and coagulation parameters could be of potential importance for the quality of donated blood products, research in a blood donation setting is lacking thus far.

Aims: We aimed to study the effect of donation-induced stress responses on coagulation parameters in whole-blood donors.

Methods: In 372 healthy whole-blood donors, multiple stress-related parameters at the moment of needle insertion were assessed. Psychological stress included donation-stress and arousal, and was assessed by a visual analogue scale. Hormonal stress was assessed by cortisol in saliva. Physiological stress included blood pressure, pulse rate, and pulse rate variability (PRV). PRV was assessed using the root mean square of successive differences (RMSSD), high frequency (HF, 0.15–0.4 Hz) power, and low frequency (LF, 0.04–0.15 Hz) power. To measure coagulation parameters, 5 ml citrated blood samples were taken from the blood diversion pouch. Using regression analyses, associations were assessed between all the obtained stress measures and coagulation parameters (PT, aPTT, FVII, FVIII, fibrinogen, vWF), reporting only significant associations ($P < 0.05$).

Results: For psychological stress, a significant positive association was found between donation-stress (mean 36.8 ± 24.6, range 0-100) and PT (mean 11.9 ± 2.6 s) ($B = 0.012$, $P = 0.025$). Calculating the potential donation-induced differences, for PT this was 1.2 s (10%). For hormonal stress, cortisol (median 3.773, 25th percentile 2.265, 75th percentile 6.304 nmol/L) showed significant positive associations with fibrinogen (mean 2.9 ± 0.6 g/l) ($B = 0.168$, $P = 0.011$), and vWF (mean 119 ± 42%) ($B = 11.376$, $P = 0.024$). Although indicating an increased coagulation with higher levels of cortisol, effect sizes cannot be estimated as cortisol was log-transformed due to skewness towards lower values. For physiological stress, pulse rate (mean 73 ± 12, range 50–113 beats per minute) showed significant positive associations with FVII (mean 0.9 ± 0.2 IE/ml) ($B = 0.002$, $P = 0.022$), and FVIII (mean 101 ± 32%) ($B = 0.540$, $P = 0.003$), and a significant negative association with aPTT (mean 31.5 ± 3.4 s) ($B = -0.035$, $P = 0.041$). Calculating the potential donation-induced differences, this results in a change of 0.1 IE/ml (14%) in FVII, of 34% (34%) in FVIII, and of 2.2 s (7%) in aPTT.

Summary/Conclusions: In conclusion, our results indicate that donation-induced hormonal and physiological stress responses induce immediate changes in coagulation parameters. The donation-induced increases in coagulation parameters seem comparable to effects induced by standardized mental stress tests.

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FACTORS INFLUENCING TOTAL PLASMA PROTEIN CONCENTRATION IN DANISH PLASMAPHERESIS DONORS

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Background: In Denmark plasmapheresis donors (PPD) are allowed to donate plasma up to ten times per year. This is considered safe with regard to stability of total plasma protein concentration (TPP). According to Danish law, measurement of TPP is mandatory at least once per year for PPD. In case of an abnormally low (<60 g/l) or high (>90 g/l) TPP result, measurement of albumin and immunoglobulins are also performed.

Aims: (i) To evaluate the current mode of monitoring TPP in PPDs, (ii) investigate possible correlations between TPP and donor characteristics, (iii) follow up on donors with abnormally low or high TPP.

Methods: For PPD with ≥1 TPP measurement, demographic parameters were extracted from the blood bank information system. For the subgroup of donors with an initially abnormal TPP, values for albumin and immunoglobulins, and, if available, TPP from the following donation were also extracted.

Results: At least one TPP measurement was available for 4,438 donors (56% male, average age 38 years, 10- and 90-percentiles: 22–57 years). For 3,580/4,438 (81%) TPP was measured prior to the first plasma donation. Average TPP was 77.0 g/l (10- and 90-percentiles: 70.4–83.7 g/l). Abnormally low or high TPP was detected in 69/4,438 (1.6%) of donors. In 35/69 (51%) of cases TPP was <60 g/l (mean 53.4 g/l); in 34/69 (49%) TPP was >90 g/l (mean 93 g/l).

There were small but significant correlations between TPP and age (-0.09 g/l/year, $P < 0.0001$) and TPP and previous allogeneic donation; the average TPP of PPDs who previously donated whole blood was 1.85 g/l lower than for PPDs who never donated whole blood ($P = 0.0002$), while the average TPP of PPDs who previously donated plasma was 0.67 g/l lower than for first time PPDs ($P = 0.0026$). The average TPP in females was 0.46 g/l higher than the average in males ($P = 0.0066$). There was no association between TPP level and ABO blood group ($P = 0.09$).

Of the 69 donors with a low or high TPP, 35/69 (51%) had a follow up TPP measurement. In 26/35 of PPDs with repeated measurements initial TPP was low; 23/26 (88%) of these PPDs had a TPP within the normal range at the next donation (median time between donations 4 months, range 1-13 months). In 13 donors with low TPP, albumin and immunoglobulin measurements showed low albumin in 10/13 (77%) and low IgM in 5/10 (50%). Having an abnormally low TPP correlated with age (donors with low TPP were on average 6.3 years older than those with normal TPP, $P = 0.0048$), but not with gender, age, previous allogeneic donation, or ABO group.

Summary/Conclusions: Abnormally low or high TPP concentrations were only seen in a small fraction of donors and had spontaneously normalized at the time of the next donation in many PPDs. There were small but significant correlations between TPP and age and with a history of previous allogeneic donation. Women had a mean TPP of almost 0.5 g/l higher than TPP concentrations in men.

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HIGH FERRITIN IN BLOOD DONORS

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Background: Ferritin is a good parameter when you want to prevent iron deficiency in regular blood donors. The question arose regarding blood donors with high ferritin levels. Are they ill with unrecognized infection or cancer? Is it alcohol? Or do they have genetic determined increased uptake?
Aims: To identify the reasons for high levels of ferritin in blood donors, in a collaboration between Centre for Donor Hemoglobin and Iron, Department of Hematology and Department of Genomic Medicine.
Methods: Between January 30th 2012 and March 24th 2014 49 (f = 6, m = 43) consecutive donors were included prospectively. One additional male had initial HFE testing done (he was heterozygous for HFE282), but did not want further investigations. Inclusion criteria were either ferritin value above 1000 μg/l or repeated high ferritin with at least one value above 500 μg/l. These donors were offered relevant clinical examination including laboratory tests and genomic analysis for mutation in HFE, HFE2, HAMP and TFR2.
Results: In all the 49 donors included, the hemoglobin concentration was above the limit for donation (12.5 and 13.5 g/dl in female and male donors respectively) and all were accepted for donation. Mean and range for age was 41 (21–63) years for the men and 46 (22–66) years for the women. The median and range for ferritin at inclusion was 539 (389–1370) μg/l for the men and 433 (354–550) μg/l for the women. Median and range for number of donations was 5 (5–100) for the med and 21 (4-115) for the women. Forty (82%) were positive for one or more mutations and 9 (18%) were negative. Two (4%) of the mutation negative donors had previously unrecognized chronic gut and lung infection respectively. One donor of Korean origin had clinical hemochromatosis, but was negative for the mutations investigated for in this study. One donor heterozygous for HFE 63 reported high alcohol consumption. Seventeen had classical HFE hemochromatosis mutations, with 10 homozygous for HFE282 and 7 compound heterozygous for HFE 282 and 63. All mutations are shown in table 1.
Summary/Conclusions: Among 49 blood donors with ferritin > 500 μg/l, 82% had mutations relevant for iron-uptake. The study only caused 4% of these donors, namely the two donors with chronic inflammation, to be deferred. In general this group of donors with high ferritin tolerates regular donations well and donors with hemochromatosis mutations may tolerate donation 4 times/year or more.

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ENHANCED DONOR ELIGIBILITY ON TRIMA ACCEL VERSION 7.0

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Background: Improving platelet apheresis productivity enables blood centers to maintain adequate inventory of a short shelf life product and improves operational cost effectiveness. Trima Accel (Terumo BCT, Lakewood CO) devices were upgraded to version 7.0, which has been designed to enhance donor eligibility through a modified post platelet count algorithm and improved procedural management.
Aims: To evaluate the impact of the modified post platelet count algorithm and procedural management changes on donor eligibility for platelet components.
Methods: This was a retrospective study consisting of 417 procedures from August 24, 2016 through September 30, 2016 on Trima Accel version 6.0 (Control) compared to 600 procedures between October 24, 2016 and January 16, 2017 on Trima Accel version 7.0 (Test). Trima Accel procedural data was captured using the Cadence System (Terumo BCT, Lakewood CO). The following targets and product definitions were used for both the Control and Test arms of the study: 3×10^{11} is a considered single, 5 & 6×10^{11} a double and 9×10^{11} a triple.
Results: A statistically significant change was observed in the donor base between the Control and Test periods, that would bias the results in favor of the Control. During the Control period donor total blood volumes were 5484 ml versus the Test which was 5,217 ml (P value < 0.001). Additionally, the Control platelet count was also higher 266,000 versus 260,000 (P value < 0.012). During the Control period of the study, donors qualified at the following rates, single platelets 5.3%, double platelets 66.4%, and triple platelets 28.3%. During the Test period of the study, donors qualified at the following rates, single platelets 2.3%, double platelets 40.2%, and triple platelets 57.5%. In the Control period, the average offered platelets per procedure was 2.23 and in the Test period it increased by 14% to 2.55 (P value <0.001). The most significant contributor to this increase was the triple platelet eligibility increasing by 103%.
Summary/Conclusions: Software modifications made to Trima Accel version 7.0 resulted in an increase in donor eligibility for multiple platelet collections. The implementation of this software may enable blood centers to collect more platelet components per procedure which contributes to improved blood center cost effectiveness and maintaining an adequate inventory.

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Abstract has been withdrawn.

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A RETROSPECTIVE ANALYSIS OF THE PRODUCTIVITY OF MCS+ UPP-999 AND C-LDP (HAEMONETICS) AND AMICUS 3.21 SINGLE AND DOUBLE DOSES (FRESENIUS KABI)

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Background: Blood cell separators (BCS) MCS+ and Amicus are automatic apheresis systems that separate and collect any combination of platelet products, plasma and concurrent red blood cells from donors, by means of discontinuous flow (MCS+) or continuous flow (Amicus3.21). Both systems reduce leukocyte load with continuous filtration.
Aims: To obtain platelets and plasma we use C-LDP protocol without additive solution with MCS+, and UPP-999 protocol in MCS+ and Amicus3.21 system with automated preparation of platelet additive solution (PAS) stored platelets. We analyzed the procedures with both CS between November 2012 and January 2017, in order to compare MCS+ system and Amicus3.21 single & Double Doses (DD), to evaluate the productivity of two BCS to obtain plasma and platelets.
Methods: We retrospectively analyzed 1843 procedures: 743 with Amicus3.21 (651 single (AS) and 92 DD) and 1100 with MCS+ (321 with UPP (MU) and 779 with C-LDP(ML)), performed with apheresis donors to obtain platelets concentrates stored with/without PAS and additional plasma. Gender and age were evaluated. Weight, height, blood volume, volume processed, platelets, Hemoglobin and hematocrit Predonation (Coulter LH750 & Dx H800), residual leukocytes, process time, platelet concentrate volume and plasma volume were measured. Adverse reactions and problems with both BCS were assessed. Data was recorded in a database (Excel 2010) and statistical analysis was done with MedCalc 12.2.1.0. P value <0.01 was considered as statistically significant.
Results: There were no statistical differences in age, weight, height, Hemoglobin, Hematocrit and white cells, between the different groups, and by different gender. However Platelet count predonation (10^9/l) was distinct between AS (240565.75 ± 39539.34) & ML (256928.97 ± 33455.25) with DD (291347.83 ± 36258.36) & MU (290498.44 ± 41562.84) as more platelet load in donor is necessary to minimize the process time with the MU and DD procedure. There were significant differences in: process time (minutes) (AS (54.36 ± 7.87); MU (55.86 ± 9.53); ML (58.52 ± 7.40) & DD (73.03 ± 11.53); plasma volume (ml) AS (318 ± 14.08) versus MU (223.66 ± 8.9) & ML (215.48 ± 14.51); blood volume processed (ml) MU (2156.82 ± 284.52) vs ML (2433.39 ± 283.72), AS (2641.49 ± 365.64), DD (3701.95 ± 481.36); platelet concentrate volume (mL) (AS (311.39 ± 7.30) & DD (309.3 ± 9.31) vs MU (275.64 ± 14.09) & ML (239.78 ± 22.88); platelet yield ($\times 10^{11}$) AS (3.94 ± 0.72) vs ML (3.47 ± 0.55), MU (3.40 ± 0.58) & DD

(3.26 ± 0.50); additive solution (ml) AS & DD (per unit) (232.97 ± 23.52) vs MU (192.70 ± 9.93) and residual leukocytes (10^6/U) AS (0.10 ± 0.15) & DD (0.08 ± 0.18) vs MU (0.33 ± 0.34) & ML (0.22 ± 0.30). Adverse reactions related to citrate, were more frequent in the ML, MU & DD groups (2.69% 2.17% & 2.18%) than in AS (1.36%). All these mild adverse reactions were successfully resolved with oral calcium. Venous rupture was more frequent with Amicus (0.40%) than MCS+ groups (0.27%). Problems with BCS operation were more frequent with MU group (6.23%) than ML (2.69%) and AS or DD group (2%).

Summary/Conclusions: More platelets yield and plasma volume in less time was obtained with Amicus 3.21 in comparison with the MCS+, with less adverse reactions and better leukoreduction. Group DD obtained more units of platelet concentrates with better cost-efficiency. However MCS+ allow us to perform apheresis in blood donation mobile units. C-LDP and UPP-999 with MCS+ and Amicus 3.21, single or DD, collects platelets and plasma complying with the European Guidelines and Spanish CAT rules.

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EVALUATION OF TRIMA ACCEL VERSION 7.0 AUTOFLOW

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Background: Trima Accel (Terumo BCT, Lakewood CO) devices were upgraded to version 7.0 in conjunction with Terumo BCT's accessory T-Cuff. The software upgrade includes several enhancements, of particular interest being AutoFlow. This feature is intended to reduce access related alerts by making proactive adjustments to flow rates based on access flow or system pressure issues detected by the Trim Accel device. In addition, the accessory T-Cuff (pressure cuff) is designed to encourage donor squeezing and ensures pressure up to 40 mmHg on the donor's vein.

Aims: To evaluate the impact of AutoFlow and T-Cuff on the frequency of low pressure access alerts that require operator intervention.

Methods: This was a retrospective study consisting of the analysis of 2,653 procedures from January 1, 2016 to September 30, 2016 on Trima Accel version 6.0 (Control) compared to 556 procedures from October 24, 2016 to January 16, 2017 on Trima Accel version 7.0 with the T Cuff (Test). Trima Accel procedural data was captured using the Cadence System (Terumo BCT, Lakewood CO).

Results: During the Control period the average number of access alerts was 2.01 per procedure whereas during the Test period the average number of access alerts was 1.02 per procedure (P value <0.001). The number of procedures with 0 access alerts increased in the Test arm by 70.3%. Furthermore, the percentage of procedures with greater than 10 access alerts decreased from 3% to 4%.

Summary/Conclusions: The implementation of Trima Accel version 7.0 resulted in a significant reduction in procedural alerts. Reducing operator interventions enables staff to spend more time with donors and to complete additional duties on the donor room floor.

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FREQUENCY AND REASONS OF DEFERRALS IN VOLUNTARY NON-REMUNERATED BLOOD DONORS IN KARACHI, PAKISTAN – SINGLE CENTRE DATA

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Background: In Pakistan, the high prevalence of HBV and HCV is due to transfusion of unscreened blood, improper sterilization of invasive medical devices used in surgery and reuse of syringes by healthcare providers (WHO). There is a lack of proper screening of donors prior to donation, resulting in transfusion of unsafe blood from high risk individuals.

Aims: Identification of the major reasons of donor deferral after implementation of detailed pre-donation screening, to exclude high risk individuals from the voluntary non-remunerated blood donor (VNRBD) pool.

Methods: Screening of voluntary blood donors was performed by 25 questions and a standard physical examination in the first centralized blood centre at The Indus Hospital Blood Centre, Karachi Pakistan. This included standard questions regarding donor wellness, high risk behaviour, health history, haemoglobin levels and a brief physical examination. In addition, a question regarding use of injections in the last ninety days was included due to the reported practice of reuse of syringes. Data was collected over a period of 37 months (Dec 2013 to Dec 2016) during blood drives.

Results: A total of 54,292 VNRBD, comprising of 89% males (48,551) and 11% females (5741), underwent screening by the questionnaire and physical examination. Out of which, 37,240 (68.6%) were drawn; the remaining were either deferred or left without donating blood despite successfully being screened. Among these visited donors, 13,057 (24%) were deferred, 10,212 (78%) males and 2845 (22%) females. The deferral rate for females was 49.6% compared to 21% for males.

In males, 36% (3,699) of deferred were due to "Use of Injection", followed by 11% (1,171) "Low Haemoglobin Levels" and 10% (1,048) due to "Not Feeling Well". In females, 64% (1809) were deferred due to "Low Haemoglobin Levels", followed by 9% (263) "Use of Injection" and 6% (169) due to "Menstrual Period".

In factories, religious seminaries (madrassas), corporate offices and higher education institutions, 9.2%, 6.8%, 6.3% and 5.2% of the total visiting donors respectively were deferred due to "use of injections".

Donors deferred due to low haemoglobin levels were 8.4%, 4.7% and 4.3% of total donors visiting in higher educational institutions, factories and corporate offices, respectively.

Summary/Conclusions: In our attempt to provide a safe supply of blood; deferring donors that used injections lead to a higher deferral rate compared to other studies done in this region. High percentage of deferrals due to injections across different sectors including educational institutes are indicative of healthcare habits and lack of awareness amongst the masses. Furthermore, high deferral due to low haemoglobin is indicative of poor nutritional status in females.

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EVALUATION OF A NEW PLATELET APHERESIS SYSTEM

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Background: Platelet apheresis has been used since the 1970s to collect one or more units of platelets from a single donor. Technological advances over the decades have allowed development of microprocessor-controlled instruments and single use disposable kits that offer operators more precise control of procedures for donor safety and comfort, are easier to use and have higher collection efficiency. This study evaluated the AmiCORE Apheresis System (AmiCORE), developed by Fresenius Kabi. The AmiCORE uses single venipuncture, continuous-flow, centrifugal separation, with ACD-A anticoagulant and saline volume replacement to collect single and double dose platelet products.

Aims: This study was designed to assess the collection performance of the AmiCORE blood cell separator and 5-day platelet storage characteristics.

Methods: Single dose platelets were collected under IRB oversight at three institutions from 31 normal, healthy and consenting donors who met appropriate donation standards. An anticoagulant ratio of 10:1, with a citrate infusion rate of 1.25 mg/kg/min, was used. The targeted platelet yield was 4.0. Donors were assessed pre- and post-procedure and monitored for adverse events. Platelets in 100% plasma were stored at room temperature for five days under constant agitation, in a non-DEHP, EVA-blend plastic storage container. In vitro hematology, biochemical and functional platelet quality parameters were evaluated on Days 0, 1 and 5. Residual leukocyte content was measured by flow cytometry.

Results: Donors had an average pre-procedure platelet count of 270 ± 64 × 10^3/μl and post-count of 220 ± 53 × 10^3/μl. All post-procedure platelet counts were > 100 × 10^3/μl. The mean platelet yield was 3.6 ± 1.4 × 10^{11}, with a collection time of 50 ± 12 min and collection efficiency of 75 ± 12%. No adverse events were observed. All platelet products met the EU standard of < 1 × 10^6 residual leukocytes, with a mean WBC count per component of 0.13 ± 0.13 × 10^6, and a maximum count of 0.4 × 10^6. Mean pH (22°C) was 7.46 ± 0.09 on Day 1, 7.46 ± 0.11 on Day 5. The lowest pH on Day 5 was 7.14. Mean HSR was 73 ± 17% on Day 1, 63 ± 13% on Day 5. Extent of shape change was 33 ± 8% on Day 1, 27 ± 8% on Day 5. CD62 was 26 ± 20% on Day 1, 35 ± 11% on Day 5. Swirling was seen in 100% of platelet products on Day 1 and Day 5. Glucose, lactate, pO_2, pCO_2, bicarbonate and LDH were at expected levels on Day 1 and Day 5.

Summary/Conclusions: The new, single-venipuncture AmiCORE blood cell separator collected process-leukocyte depleted platelets safely and efficiently. Platelet *in vitro* assays demonstrated maintenance of *in vitro* platelet quality measures during 5 day storage.

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COLLECTION OF BLOOD COMPONENTS BY APHERESIS IN THE RUSSIAN FEDERATION

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Background: The use of apheresis systems is productive procedure for obtaining of blood components with high efficiency and safety, but implementation of apheresis in practice requires advanced technical equipment. However, this allows to use the donor's potential optimally and to reduce donor exposure in recipients. Determination of the apheresis implementation properties in the national blood service is a significant problem.
Aims: The aim was to study the organizational aspects of the blood components collection by apheresis in the blood service establishments in the Russian Federation.
Methods: Performance indicator analysis of blood service establishments in the Russian Federation represented in sectoral statistical observations over the period 2013–2015, and the calculation of indices characterizing the level of development of the apheresis donations were conducted. The analytic data are presented according to the Russian Federation administrative division into federal districts (FD).
Results: Plasma for transfusion (clinical use) and for fractionation into plasma proteins is collected by apheresis donations. On average 37.8% (range 34.7–39.8%) of plasma was collected by apheresis in blood service establishments in the Russian Federation for the period 2013–2015. The largest percentage of plasmapheresis plasma was observed in the Central FD (48.0%), the lowest – in North Caucasus FD (21.1%). The volume of plasma collected by apheresis per 1,000 inhabitants was 2.02 L in 2015 and varied considerably in regions. In the Central FD, plasmapheresis plasma was 2.70 L per 1,000 inhabitants, while in the North Caucasus FD it was 0.65 L. On average 63.5% (range 56.1–67.9%) of platelet concentrates were collected by apheresis in 2013–2015. In the Central FD 82.7% of platelet concentrates were obtained by apheresis, while in the Southern FD – 46.4% in 2015. The percentage of red blood cells (RBC), collected by apheresis, varied from 0.43% to 0.72% (median – 0.55%). The largest percentage of RBC apheresis was observed in blood service establishments in the North-West FD; in the Southern FD and North Caucasus FD these apheresis procedures was not used for RBC collection. Granulocyte apheresis donations are rare procedures in the blood service establishments in Russian Federation. In the future, the number of apheresis donations will increase in blood service. This will require the monitoring of adverse reactions and complications at donors and training of medical personnel.
Summary/Conclusions: For the period 2013–2015, on average 37.8% of plasma, 63.5% of platelet concentrates and 0.55% RBC were collected by apheresis in blood service establishments in the Russian Federation. The significant variability of the degree of implementation of apheresis in different regions of the country is stated. The obtained data are important for future planning of blood service activity.

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COLLECTION EFFICACIES OF A NEW APHERESIS SYSTEM FOR SINGLE DOSE AND DOUBLE DOSE PLATELET COLLECTION

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Background: New generation apheresis systems enable a multiple unit collection of platelets from the one eligible donors in a single procedure. The advantages of this procedure are maximization of donor resources, risk reduction of transfusion transmitted diseases and reduction of production costs.
Aims: The aims of this study is to evaluate the performance and collection efficacy of the AmiCORE Apheresis System, Fresenius Kabi AG, Germany for single dose and double dose plateletpheresis. The platelet quality characteristics and donor safety were also evaluated.
Methods: Twenty-eight repeated donors were recruited for plateletpheresis. Single dose platelets (SDP) and double dose platelets (DDP) were collected in 100% plasma for the target yield of 2.8×10^{11} and 5.6×10^{11}, respectively. Donor pre- and post-donation parameters, procedure and platelet quality characteristics were measured. Donor reaction were observed.
Results: Prior to platelet collection, Donors for SDP and DDP had average platelet count of $262 \pm 31 \times 10^3/\mu L$ and $325 \pm 77 \times 10^3/\mu l$, respectively. None of the donors have post-donation platelet count less than $100 \times 10^3/\mu l$. Red cell loss from SDP and DDP collection averaged 20 ± 2 ml. Collection times averaged 44 ± 6 min for SDP and 70 ± 18 for DDP. The SDP collections had an average platelet collection efficiency of $76.1 \pm 10.2\%$, producing a total average platelet yield of $2.77 \pm 0.36 \times 10^{11}$. The DDP collections had an average platelet collection efficiency of $74.5 \pm 10\%$, producing a total average platelet yield of $5.5 \pm 0.7 \times 10^{11}$.The average actual to targeted platelet yield ratios were 0.99 ± 0.13 for SDP and 0.96 ± 0.12 for DDP collection. All of SDP and DDP had residual white blood cells $<1 \times 10^6$/unit. No adverse events were reported from any donors.
Summary/Conclusions: The efficiency and safety of SDP and DDP collection by the AmiCOPRE Apheresis System has been revealed in this evaluation. Leukoreduced platelets had acceptable characteristics and passed international standard requirement.

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NEW SINGLE NEEDLE PLATELET APHERESES SEPARATOR AMICORE: FIRST EXPERIENCE REPORT

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Background: Platelet collection by means of apheresis is one of two main pillars of platelet production. Using a single needle, continuous processing technique AMICORE (Fresenius-Kabi, Lake Zurich, IL, USA) is a new device for platelet production. Based on the successfully used Amicus technology, but improved in size, user friendliness and donor comfort, AMICORE has been CE marked and is ready for use in Europe.
Aims: Aim of the study was to investigate performance of platelet collection on the new device and to check if the produced units meet the European quality requirements for platelet content and leucocyte contamination without additional filtration.
Methods: 8 platelet collections were performed with a single needle disposable to collect either a single unit(SU) (3.0×10^{11} platelets per unit, six procedures) or a double dose unit (DU) (6.0×10^{11} platelets per unit, two procedures). We report here performance data (duration, draw and return speed, platelet concentration, yield and adverse events). Data are given as mean values.
Results: Seven male and one female donors donated six SU and two DU. No donor reported adverse events, even not citrate triggered symptoms. Counted vs programmed yield was $4.22*10^{11}$ vs $3.75*10^{11}$ for all procedures, $3.31*10^{11}$ vs $3.0*10^{11}$ for SU and $6.94*10^{11}$ vs $6.0*10^{11}$ for DU. Platelet concentration of all units was $1085*10^3/\mu l$ and all units were resuspended in 100% plasma, 300 ml for SU and 600 ml for DU. Procedure time in minutes was 67 for all procedures, 95 for DU and 58 for SU. All procedures were performed at a mean draw rate of 103 ml/min and mean return rate of 97 ml/min. Precounts of donors was $262*10^9$/l. Automatic adjustment of flow rates avoided flow alarms and no intervention of operators was necessary. Leucocyte reduction was performed in-process without the use of leucocyte depletion filters and all units contain less than $1.0*10^6$ leucocytes ($0.07*10^6$). Within all procedures no alarm occurred and no failure was reported by the system.
Summary/Conclusions: Platelet collection on the AMICORE was well tolerated by all donors; flow rates were also accepted and forced no interaction by the operator. SU as well as DU fulfilled quality requirements regarding the platelet content per unit and the leucocyte contamination.

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SPECTRA OPTIA – INITIAL SEPARATION AT THE DEPARTMENT OF TRANSFUSION MEDICINE UNIVERSITY HOSPITAL OLOMOUC

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Background: In November 2015, Spectra Optia Apheresis System was introduced at the Apheresis Centre of Department of Transfusion Medicine at University Hospital Olomouc. It is a mobile, automated separator of blood components that uses centrifugation and optical detection through Automated Interface Management (AIM) system for therapeutic apheresis procedures and cell collection procedures.
Aims: To analyse the quality of obtained products.
Methods: Several initial collections of selected procedures were made from December 2015 to December 2016. These were: donor platelet collection, red blood cell exchange, continuous mononuclear cell collection, and granulocyte collection. All the procedures, except for platelets separation, require double-needle access.

Results: There were 70 (8 × 1TU, 40 × 2TU, 22 × 3TU) procedures of donor platelet collection through Platelet collection-TROMBO. These were conducted into REF 10,400 collection set, with 3 TU collections an accessory collection bag for platelet storing REF 70,030 was used as well. Quality parameters of the products met the requirements, however, it is not possible to collect platelets into substitute solution with this device.
The red blood cell exchange (RBCX) was done with 3 patients (1 patient with secondary polycythaemia and 2 patients with familial erythrocytosis). The collection set REF 10,220 was used for the depletion of red blood cells. From 413 g to 616 g of packed red blood cells with complete volume replacement with saline solution was collected from the patients, the procedures took 35–40 min. The limitation of double-needle access was most perceptible with this type of collection.
The procedure of granulocyte collection (PMN) was done 20x with donors who were stimulated with corticoids into the collection set REF 10,300, 10% Voluven solution was used as HES. The parameters of procedures were as follows: 6,000 ml of whole blood were processed, procedure time from 96 to 114 min, inlet flow rate 65 ml/min, collection flow 3.9–4.8 ml/min, collection preference 28–40. Quality parameters of granulocytes from apheresis: leukocytes 15.4–50.1 × 10^9/l, neutrophils 11.2–42.9 × 10^9/l, Hb 52–83 g/l, Hct 0.13–0.23, granulocyte 0.43–1.79 × 10^{10}/TU, volume of the product from 402 to 483 ml.
The continuous mononuclear cell collection (CMNC) procedure was done with ten donors for the collection of mononuclear cell concentrate for an external customer (biotechnological company) for study and research. The collection set REF 1,0300 was used for this procedure. Ten leukapheretic products were taken (Mononuclear cell concentrate), volume of the product from 112 ml to 130 ml. The parameters of procedures were as follows: 9,000–10,200 ml of whole blood were processed, procedure time from 157 to 183 min, inlet flow rate 65 ml/min, collection flow 0.8 ml/min, the Inlet:AC Ratio was set to 10:1. All products met the requirements for the volume of mononuclear cells >4 × 10^9/TU.
Summary/Conclusions: Spectra Optia suitably supplemented the portfolio of apheresis devices of our department. We welcomed the possibility of collecting donor platelets that increases the efficiency of the device even though platelets can be separated only into plasma. The need for double-needle access limits the depletion of red blood cells in patients. There was no problem with the vein access with the rest of the collection procedures.

Donor adverse events

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IMPROVING SAFETY AND ENHANCING THE DONATION EXPERIENCE FOR APHERESIS DONORS

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Background: Australia has seen an 80% increase in the number of plasmapheresis collections since 2010. This has required very rapid growth in the apheresis donor panel over the past 7 years. Citrate reactions, caused by a reduction in the concentration of ionised calcium in the blood on exposure to citrate anticoagulant used in apheresis procedures are commonly reported by platelet donors, and less often by plasma donors. Oral calcium supplements (effervescent tablets containing 1,000 mg calcium gluconate) have been provided to symptomatic donors. The tablets are unpalatable, are not infrequently associated with gastrointestinal side effects, and most donors refuse them. The return rate of donors experiencing citrate reactions is lower than those who do not experience citrate reactions.
Aims: The aim of this initiative was to improve apheresis donor safety by proactively managing donors to minimise citrate reactions thereby enhancing the donation experience.
Methods: All donor adverse reactions are reported via a centralised database. Reports of citrate reactions were reviewed by donation type (plasmapheresis, single and double plateletpheresis) and the incidence of reactions calculated for a period of 2 years. A palatable formulation of calcium was identified (a chewy, spearmint flavoured lozenge containing 330 mg calcium). Following a brief education campaign with donor centre staff, a new standard operating procedure was introduced, requiring that staff inform donors that "to reduce the frequency of metallic taste and tingling in the mouth, hands and feet, the Blood Service recommends that all apheresis donors have 3 lozenges before their donation". Donors were able to refuse; in addition, donors were offered additional lozenges during donation if they experienced citrate related symptoms.
The incidence of citrate reactions was monitored weekly for the 12 weeks following introduction of the new process and the incidence of citrate reactions was compared for 24 weeks prior to the change and 12 weeks following the change in approach.
Results: In the 24 weeks prior to the introduction of the new procedure there were 251,135 plasmapheresis collections with 1 966 citrate reactions reported (incidence of 0.78%); during the 12 weeks after the introduction of the new procedure there were 145,001 plasmapheresis collections associated with 872 citrate reactions (incidence 0.6%; RR 0.7682, 95% CI 0.7095–0.8317, P < 0.0001). In the 24 weeks prior to the implementation of the new procedure, there were 15,382 plateletpheresis collections with 1013 citrate reactions reported (incidence of 6.59%). 12 weeks after the introduction of the new procedure, there have been 7,248 plateletpheresis collections with 342 citrate reactions reported (incidence 4.72%, RR 0.7165, 95% CI 0.6359–0.8073, P < 0.0001).
Summary/Conclusions: Citrate reactions are unpleasant for apheresis donors and can result in donor attrition. Their occurrence can be significantly reduced by the prophylactic use of palatable oral calcium supplements. The key to donor acceptance of this intervention has been achieved through staff education and provision of a standard script to encourage donor acceptance.

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11% OF FINNISH BLOOD DONOR HAVE IRON DEFICIENCY BUT THEIR SELF-REPORTED SUBJECTIVE HEALTH IS NOT AFFECTED

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Background: Iron deficiency is a known long-term undesirable consequence of blood donation. Only a few studies on blood donor iron stores have measured soluble transferrin receptor (sTFR). Iron deficiency has been connected to adverse health effects, but not many studies have examined the relationship between iron deficiency and donor's subjective health.
Aims: To estimate the proportion of blood donors with iron deficiency (ID) and if ID affects their subjective health.
Methods: Fin Donor 10 000 is an on-going prospective study observing the relationship between health and iron stores in active Finnish blood donors.
1,711 blood donors (1,051 women, 660 men) enrolled the study between 18.5.2015 and 30.6.2016 in the capital region of Finland. Venous hemoglobin (vHb), ferritin and sTFR were measured within 24 h of sampling. Abnormal sTFR value was defined as over 5 mg/l in men and over 4.4 mg/l in women; abnormal values indicated ID.
Donors participating the study were asked to fill in an internet questionnaire about their subjective health, eating habits and use of iron and vitamin supplementation.
Results: 13% of women and 7% of men had ID. All donors with ID had lower ferritin (below 12 mg/l) and lower mean vHb, than those without ID. See Figure 1 for distribution of vHB and ferritin values according to sTFR strata.
Questionnaire data was available from 1,687 donors. See sex-specific strata on table 1. Statistical testing (Fisher's Exact test, 2-sided) was performed between the iron deficient (IDG) and the non-iron deficient (NIDG) groups. Subjective health (general health either "excellent" or "very good") did not differ between donor groups (IDG 63.3%, NIDG 60.6%), P = 0.464. Day-time tiredness (tired during the day "always" or "often") did not differ between the donor groups (IDG 15.1%, NIDG 13.9%), P = 1. Donors, who reported having low hemoglobin value (women <125 g/l, men <135 g/l) during lifetime, were more common in iron deficient group (IDG 57.2%, NIDG 40.0%), P < 0.0001.
Summary/Conclusions: Proportion of ID in this study corresponds the previously reported rates in other donor populations. A significant portion of the donors reported hemoglobin values below the sex-specific donation limit, which is partly explained by the unequal sex division in the study population. Association between sTFR and ferritin indicates that ferritin measurement alone might be utilized for ID screening in blood donors.
Iron deficiency did not have an influence on the subjective experience of health or day-time tiredness in blood donors. Previous reports have connected ID to adverse health effects in blood donors, the present study does not support those findings.

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ASSOCIATIONS OF HEALTH STATUS WITH DONATION CESSATION AND INTENSITY: AN ALTERNATIVE PERSPECTIVE ON THE HEALTHY DONOR EFFECT FROM DONOR INSIGHT

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Background: In donor health research, the 'Healthy Donor Effect' (HDE) often hampers study results and their interpretation. This refers to the fact that donors are a

selected 'healthier' subset of a population due to both donor selection procedures and self-selection. Donors with long versus short donor careers, or with high versus low donation intensities are often compared to avoid this HDE, but underlying health differences might also cause these different behaviours.
Aims: Our aim was to estimate to what extent a donor's health status associates with donation cessation and intensity.
Methods: All active whole blood donors participating in Donor InSight (2007–2009; 11,107 male, 12,616 female) were included in this prospective cohort study. We performed Cox survival and linear regression analyses to assess whether self-reported health status, medication use, disease diagnosed by a physician and recent visits to a general practitioner (GP) or specialist were associated with (time to) donation cessation and donation intensity.
Results: At the end of 2013, 44% of the donors had stopped donating. Donors in self-rated good health had a lower risk to stop donating compared to donors in poor or neutral health. Medication use, disease diagnoses and visits to a GP were associated with an increased risk to stop donating, even after adjusting for age, smoking and number of donations. Both men and women reporting good health were more likely to donate with a higher intensity.
Summary/Conclusions: Donors with a good health status were less likely to stop donating blood and tended to donate blood more often than donors with a worse health status. This implies that the HDE is an important source of selection bias in studies on donor health and this includes studies where comparisons within donors are made. This HDE should be adjusted for appropriately when assessing health effects of donation.

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INCIDENCE OF BLOOD DONATION COMPLICATIONS USING THE NEW ISBT/IHN/AABB STANDARD DEFINITIONS AND CLASSIFICATION SCHEME

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Background: After publication of a new set of standard definitions and classification scheme of blood donation complications (DC) by a joint working group of the International Society of Blood Transfusion (ISBT), the International Haemovigilance Network (IHN) and AABB, we revised and enhanced our donor haemovigilance process.
Aims: To provide data on incidence of DCs using the new ISBT/IHN/AABB standard definitions and classification scheme and to illustrate the impact of implementing a new donor haemovigilance process in a blood center.
Methods: Only moderate and severe DCs were to be reported by the blood center (BC) personnel. The new system was implemented in two phases: (1) addition of a question to our electronic donor health questionnaire (eDHQ) inquiring about DC at last donation in May 2015 and (2) development of a DC management manual including the new ISBT-IHN-AABB definitions and classification scheme; a new reporting form and enhancement of database on October 12, 2016. All severities of DCs had to be reported since phase 2. eDHQ DCs that matched DCs reported on a form were excluded. Rates of DCs are presented per 100 donations for the period 12 October 2015 to 11 October 2016. Chi-square tests were used to compare rates.
Results: In the 12-month period following the implementation of our new donor haemovigilance process a total of 20,244 DCs were reported out of 300,462 donations for a rate of 6.74 per 100 donations. 11,979 were to whole blood donations (WB) and 8265 to apheresis donations (A) for respective rates of 5.30 and 11.08. A total of 2040 arm complications were reported for rates of 0.47 (WB) and 1.31 (A). 12,810 vasovagal reaction (VVR) were reported for rates of 4.82 (WB) and 2.57 (A). For VVR with loss of consciousness (LOC) (n = 753) rates were 0.30 (WB) and 0.10 (A). Rates of VVR were significantly higher for female donors (7.00 vs 2.42 for males, $P < 0.001$) and the same was true for VVR with LOC (0.41 vs 0.14, $P < 0.001$). There was a significant declining trend of VVR with age: 18–22 (13.53), 23–29 (6.86), 30–39 (4.38), 40–49 (2.56), 50–59 (1.59), 60–70 (1.26), 71+ (0.80). There were 5,354 citrate reactions (99.7% mild) for a rate of 7.17 per 100 donations. An average of 86 DCs per month was reported before our new reporting system. Phase 1 increased reporting to 256 per month. Phase 2 increased reporting to an average of 1,687 DCs per month, a 19.6-fold increase compared to the previous system.
Summary/Conclusions: Incidence of DC is far from being trivial. Implementation of a direct question to donors on DC at last donation significantly increased the number of reports (by 3 fold). Reporting of all DCs is important to better estimate the true rates of DCs after a blood donation and to provide more sensitive data that will help evaluate the implementation of preventive measures much more rapidly.

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INCREASED INTER-DONATION INTERVAL IS ASSOCIATED WITH INCREASED DONOR HEMOGLOBIN AND FERRITIN LEVELS

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Background: Frequent blood donations results in decreased/absent iron stores in donors with normal hemoglobin (Hb) levels. Based on the ferritin levels (ferritin) in a subset of our whole blood donors (WBD), we increased inter-donation interval from 8 to 12 weeks to mitigate the loss of iron stores due to frequent blood donations.
Aims: Analyze WBD ferritin and Hb levels blood donors in both periods after increasing inter-donation interval.
Methods: Ferritin (Nr: Female = >11 ng/ml, Male = 24 ng/l) was measured in 1,000 WBD from August-December 2012. Inter-donation interval was increased to 12 weeks in May 2013 for all WBD. Ferritin in another 1,000 WBD was measured from Jun-September 2014. Donation history in the prior 12 and 24 months, pre-donation hemoglobin, red meat intake, smoking, vitamin or iron supplements was assessed. Data was analyzed using the Wilcoxon rank sum test and Chi square test, as appropriate, with significance defined as P-value >05.
Results: 361 and 272 WBD were deferred for low ferritin in 2012 and 2014 respectively (P: <0.0001). Donors with low ferritin in 2012 had donated an average 4.4 and 7.6 times and in 2014 had donated an average 3.5 and 6 times in the past 12 and 24 months (P:<0.0001). Donors with normal ferritin in 2012 and 2014 had donated an average 3 and 5 times in the past 12 and 24 months (P:<0.0001). The average Hb significantly increased from 14.2 g/dl (SD: 1.3) in 2012 to 14.4 g/dl (SD:1.2), P:0.007. The average ferritin levels significantly increased from 30.7 ng/l (SD: 31.1) in 2012 to 39.7 ng/l (SD:69.5), P:<0.0001. There was no significant change in the donor demographics in both periods. Change in ferritin levels and hemoglobin levels did not correlate with red meat intake but correlated with number of WBD in the previous two years.
Summary/Conclusions: In our study we find that number of donations in the past 24 months is a strong predictor of low ferritin in WBD and increasing the inter-donation interval may help mitigate this.

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Abstract has been withdrawn.

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SAFETY DURATION FOR REPEAT DOUBLE PLATELET APHERESIS: AN INTERVENTION STUDY IN VIETNAM

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Background: In Vietnam, the interval between two consecutive double-bag platelet collections is 4 weeks as frequent collections are anticipated to cause harmful effects.
Aims: This study aimed to compare a complete blood count (CBC) and occurrence of side effects after consecutive double-bag platelet (PLT) apheresis among 3 group with a 2, 3 and 4-week interval.
Methods: This was an intervention study conducted at Blood Bank of Blood Transfusion and Hematology Hospital. Eligibility criteria were age of 18–60 years, body weight of at least 55 kg, normal CBC with a number of platelets of at least 250,000/μl at the baseline and 225,000/μl during the follow-up. Exclusion criteria were uncontrolled chronic diseases, acute illness with fever, whole blood donation 3 months prior to the registration, and currently receiving or had received within the 2 weeks an antiplatelet therapy. Eligible PLT donors were randomly assigned to 3 groups: double-bag platelet apheresis collections with a 2, 3 and 4-week interval. All participants were followed for maximum of 10 consecutive platelet collections using Trima Accel (Terumo BCT, USA).
Results: During 12 months, a total of 338 eligible platelet donors were divided into 3 groups: 2-week group (n = 114), 3-week group (n = 110), and 4-week group (n = 114). Basic characteristics showed no statistically significant differences among 3 groups.

Total number of collections during the study period was 1693 times. The number of completed procedures was 655, 555, 483, and the average of PLT apheresis per donor was 5.7, 5.0 and 4.2 times for 2, 3, 4-week group, respectively. Total of donors who came five or more times in each group was 66 (57.9%), 49 (44.5%) and 42 (36.8%), respectively. The major reason for the dropout was coming to donation earlier or more than 2 days later than the specified date (32.5%, 48.2% and 57% among dropouts in each group, respectively).
Before each PLT apheresis procedure, all of PLT donors were tested for CBC to ensure eligibility. Median PLT ranged 286–380 k/ul, median Hb 13.8–14.4 g/dl, median WBC 6.4–7.3 k/ul, and median protein on the last collection 72.5–74.6 g/dl. Of note, proportion of those with PLT > 150 k/ul after the 1st PLT apheresis in each group was 98.1%, 93.5% and 95.4%, respectively.
The frequency of side effects among 338 PLT doors was 73.7% (Chi-square test, P = 0, of which were mild symptoms including light numbness on libs and limbs, dizziness, sweating, pale, fatigue, increased blood pressure and high heart rate.
Summary/Conclusions: We observed no statistical significant differences in occurrence of side effects among 3 groups of donors with 2, 3 or 4-week intervals. No evidence of moderate or severe adverse events were found. Pre-procedure PLT, Hb, WBC counts were often higher in the 2 shorter duration groups. Consecutive platelet apheresis of the double-bag PLT donors with an interval shorter (2 or 3 weeks) than the current duration in Vietnam (4 weeks) is a safe and efficient PLT collection strategy.

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DONOR HEMOVIGILANCE: HAVE WE GOT THE FIGURES RIGHT?

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Background: Donor hemovigilance is an important aspect for any blood transfusion facility. Based on the time of occurrence it can be classified as acute or delayed. Delayed adverse reactions occur 24 h after the donor has left the donation site. The transfusion service is aware only in case a donor informs back to the facility leading to an underreporting of the donor adverse events.
Aims: This prospective study aims to know the incidence and types of delayed donor reactions and the risk factors associated with it, amelioration of which would improve the donor return rate.
Methods: The study was conducted prospectively from May 2016 to December 2016 at the Department of Transfusion Medicine, Indraprastha Apollo Hospitals, New Delhi. One thousand blood donors who donated blood at our center during the said period were randomly selected and a telephonic call was made to enquire about the feedback and analyzed. Root cause analysis (RCA) was done to identify the risk factors associated with the adverse events. Their previous donations and any previous adverse events, if any, were also enquired about.
Results: Thirteen hundred and fifty four donors were called of which 354 (26%) did not respond to the telephonic calls. Forty-eight (13.5%) telephone numbers were incorrect and the rest did not answer the call. Of the 1,000 donors who responded, 984 (98.4%) were males and 16 (1.6%) females. The mean age group was 30 (range: 18–61 years). Four (0.4%) donors reported bluish discoloration (bruise) at the phlebotomy site. They were called back to the donation center. Of them, 2 had moderate pain for which they took medication. There was no other sensory or motor deficit. They were advised to apply thrombophob locally. The bruise faded within 4-7 days of time in all of them. On RCA it was found that the bruise was a result of manipulation of the phlebotomy site by the new phlebotomist. She was counseled and trained further to prevent any such adverse event.
Summary/Conclusions: Call back policy was effective in bridging the gap between the actual donor reactions encountered and the ones reported. It led to the RCA which strengthened our lacuna and improve our services to retain the blood donors in the pool and prevent their drop outs because of adverse events.

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DONOR INSIGHT-III: AN UPDATE OF THE DATA-COLLECTION

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Background: Blood donation results in a loss of erythrocytes that contain hemoglobin (Hb) and therefore new erythrocytes and Hb needs to be produced. Donors differ in Hb levels and Hb level recovery after blood donation. Some donors show relatively stable Hb trajectories after multiple donations, while other donors show declining trajectories. Several factors including sex, age, and season are known to influence Hb levels. Still a good explanation for the different Hb trajectories between donors is lacking.
Aims: DIS-III aims to investigate associations of lifestyle and genetic factors with trajectories of Hb levels in blood donors.
Methods: DIS-III is an observational cohort study among Dutch donors who participated in DIS-I and/or DIS-II. DIS-III was conducted between April 2014 and December 2016. Three groups of donors were invited; a group with declining and a group with stable Hb trajectories based on latent-class growth analyses and a randomly selected group of donors. An invitation letter with information brochure, reply card and a pictorial blood loss assessment chart (women only) was sent by post to 6,140 DIS-III donors. Two weeks after the invitation letter, reminder letters were send to donors from whom we received no response (n = 4,540). From two weeks after the reminder letter, donors from whom we received no response were contacted by phone (n = 3,652). These donors were called until we received a response, with a maximum of three call attempts. Non-responders of whom an email address was known also received a reminder email (n = 1,331). Donors were asked to complete two online questionnaires: (1) a general questionnaire on lifestyle, menstruation, menopause, pregnancies, health and disease and (2) an adapted food frequency questionnaire focusing on iron intake. Besides, donors were asked to provide four blood samples. In case of active donors, blood samples were drawn from the sampling pouch during a regular donation. Inactive donors were asked to visit the blood bank once to provide the blood samples through venipuncture. The blood samples were used for a complete blood count, to study hemolysis, to measure ferritin, zinc protoporphyrin and lipids and to isolate and analyze DNA.
Results: In total, 2,864 donors provided an informed consent and blood samples for DIS-III. Of these donors, 1,109 had a declining Hb trajectory, 838 had a stable Hb trajectory and 1,024 belonged to the randomly selected group. The general questionnaire was completed by 2,495 donors and the food frequency questionnaire was completed by 2,477 donors. Eventually, 2,160 donors could or would not participate because of health reasons, time constraints or they could not be reached.
Summary/Conclusions: The DIS-III data-collection has been completed successfully. Increased knowledge on genetic and lifestyle factors that distinguish between donors with different Hb trajectories after repeated blood donations will help to select donors and tailor their donation intervals in order to prevent iron deficiency and donor deferral.

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Abstract has been withdrawn.

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THE CRUCIAL ROLE OF BLOOD COLLECTION STAFF IN PREVENTING AND MANAGING VASOVAGAL REACTIONS

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Background: Applied muscle tension (AMT) and water loading (WL) reduce the risk of vasovagal reactions (VVRs). Blood collection staff play a key role in educating donors about these techniques, as donors look to staff for guidance in completing their donation safely. Moreover, staff interactions with donors improve the donation experience and enhance donor return. Despite their crucial role, little is known about how staff educate donors about VVR prevention.
Aims: This study aims to understand the knowledge, attitudes, and behaviours of blood collection staff with regard to VVR prevention. This is a critical step to implementing VVR prevention strategies and maximising donor adherence.
Methods: A mixed-methods approach was used to collect in-depth information from staff focus groups (63 participants) and document broader experiences through a national staff survey (n = 285). The focus groups explored VVR risk factors, attitudes to VVR prevention, knowledge of AMT, and suggestions for implementation. The survey assessed use of VVR prevention techniques, motivators and barriers to AMT, and communication preferences.
Results: Staff identified donors at high-risk of VVRs as being first-time, anxious, young, motivated to donate for emotional reasons, and those who donate in a group. Staff currently attempt to prevent VVRs by providing hydration education, ensuring comfortable environments and good communication with donors, and actively distracting at-risk donors during donation. Staff were clear on the importance of hydration. However, consistent with the pre-donation text message currently sent to

donors, many staff emphasised hydration prior to arrival, with fewer asking donors to use WL during their time in centre.
Most staff (71%) reported recommending AMT at least some of the time; however, the focus groups revealed some variability in why staff thought AMT should be recommended. Despite the positive effect of leg-crossing on blood pressure as a component of AMT, staff did not always recommend this and rather preferred donors to uncross their legs to improve blood flow. Staff also identified several barriers to the routine practice of AMT in the operational context. These included concerns that it would impact donor satisfaction through adding to the pre-donation wait time (to be taught AMT) and the fact that some donors did not perceive that they needed the technique. Nevertheless, staff felt the technique would be beneficial and expressed willingness to consistently recommend the technique.
Summary/Conclusions: This study highlights important information with regard to the practices blood collection staff currently engage in to prevent VVRs and the education they provide to donors. The importance of hydration is emphasised, however aspects of the timing of the hydration may not be optimal for VVR prevention. AMT is also frequently recommended for use; however, gaps in knowledge about AMT and barriers to use of AMT were identified. Planned organisational changes designed to align with the evidence-base of best practice of VVR prevention will be discussed.

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REPORT OF ADVERSE TRANSFUSION REACTIONS: EMOCOMPONENTS VIA APHERESIS VS WHOLE BLOOD IN PEDIATRIC PATIENTS

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Background: The focus on transfusion reactions requires continuous improvements of blood collection and preparation by imposing clinical assessments especially in pediatric patients where the incidence of adverse reactions is historically double with respect to adult patients.
Aims: The purpose of this study was to detect adverse reactions of emocomponents from whole blood and aphaeresis in thalassemic and oncological pediatric patients.
Methods: In 2011–2015, U.O.C. Clinical Immunology, Immunohaematology and Transfusion Medicine allocated 12.651 units of emocomponents to Pediatric Department, Università degli Studi della Campania "Luigi Vanvitelli".
Results: During this period, the incidence of adverse reactions after transfusion of packed red blood cells (pRBCs) was 0.12% (n = 6/4.684) (3 minor allergic reactions, 1 febrile episode, 2 episodes of vomiting); 0.16% after leukoreduced red blood cells (LD-RBCs) transfusion (n = 9/5.530) (7 minor allergic reactions, 1 febrile episode, 1 episode of vomiting); 0.03% (n = 4/1.296) (4 minor allergic reactions) after erythroplasmapheresis (EPA) transfusion while no adverse reaction was observed after erythroapheresis transfusion (EA) (n = 26). In addition, an incidence of 0.5% (n = 20/374) (11 minor allergic reactions, 2 febrile episodes, 2 episodes of vomiting and 5 episodes of dyspnea and bronchospasm) of adverse events after platelet concentrates (PCs) transfusion was registered while an incidence of 0.87% (n = 4/457) (4 minor allergic reactions) after transfusion of buffy-coat-derived platelet pools was reported. No adverse reaction was observed after transfusion of platelets derived by apheresis (PLT-A) (n = 0/196). Moreover, no adverse reaction was notified after transfusion of fresh frozen plasma (FFP) (n = 61), of plasma obtained via erythroplasmapheresis (P-EPA) (n = 20) or via plateletapheresis (P-PA) (n = 8).
Summary/Conclusions: As expected, the highest adverse event incidence was associated with the infusion of PCs (0.5%) while no adverse reaction was observed with any type of transfused plasma. Regard to RBCs, no relevant differences of transfusion reactions were found among EPA, LD-RBCs and pRBCs. Although preliminary data showed a low incidence of post-transfusion reactions in pediatric patients, they are encouraging to improve the aphaeresis collection by specialized staff. Given the absence of adverse reactions associated with platelet transfusions via apheresis, we advocate the need to employ these collections to preserve the pediatric patients from the immunological and infectious exposure derived from pools of different donors.

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IMPROVING DONOR SAFETY DELAYED VASOVAGAL REACTIONS ASSESSMENT IN THE LISBON BLOOD AND TRANSPLANTATION CENTRE (2011–2016)

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Background: Vasovagal Reactions (VVR) represent the most frequent category of Donor Adverse Reactions (DAR). The VVR can be classified into two groups: Immediate or Delayed; ii. With injury or without injury. According to ISBT/IHN standard for surveillance of donation complications, VVR are considered delayed when the symptoms occur after the donor has left the donation site. VVR are considered with injury when they are caused by falls or accidents in donors with a vasovagal reaction.
Aims: (i) To assess the prevalence and the ratio of delayed VVR in Whole Blood donation in Lisbon Blood and Transplantation Centre (CSTL) between 2011 and 2016; (ii) To assess the frequency of delayed VVR with and without injury; (iii) To analyze delayed VVR: donor's characteristics, location of the donation, severity and donor recovery.
Methods: A retrospective analysis of delayed VVR reported to the Portuguese Haemovigilance System (SPHv) between 2011 and 2016 has been performed to calculate the prevalence, ratio and characterization of delayed VVR. Data was recorded according to local procedures and classified using ISBT/IHN standard for surveillance of donation complications.
Results: Between 2011 and 2016, 81 VVR were reported to the SPHv with a rate of 2/10,000 donations, representing 7% of the total DAR reported (1,136). Throughout the years, this rate has been constant. Most of the reported delayed VVR were without injury (83%). The rate of delayed VVR was 1/10,000 in first-time donors and 2/10,000 in female donors. In relation to the donors age range, the rate was 6/100,000 between 18 and 24 years and 1/10,000 between 25 and 44 years. Regarding the donation site, the majority of the delayed VVR occurred in mobile collection sessions (97%). Only 20% of reported delayed VVR were serious adverse reactions, but in 11% of the cases the donor needed hospitalization.
Summary/Conclusions: Notification of donor adverse reactions is an important source of information and knowledge that, through analysis of data, allows the implementation of preventive and corrective measures in order to improve blood donation safety.
The results underlined that the prevalence of delayed VVR in CSTL has been constant over the last years and it is almost residual when compared with the total amount of whole blood collections. Nevertheless note that the real amount of reactions occurred in the reviewed period is unknown, hence the post donation information procedure has been implemented. The presented results show the education and training of all the professionals involved in the whole blood collection process has been crucial in the ability to respond to complications of blood donation.

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HEMOGLOBIN, ANEMIA AND RETURN TO BLOOD DONATION IN PROSPECTIVE WHOLE-BLOOD DONORS IN THE FRENCH WEST INDIES IN 2015

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Background: Blood donor deferral rates are high in the French West Indies, especially due to anemia (hemoglobin levels below the required threshold of 12 g/dl for women and 13 g/dl for men). Most of the donors and recipients of blood donors in the French West Indies are of African-Caribbean origin. This population has a number of specific characteristics, including a high frequency of hemoglobinopathies and a distribution of blood group antigens very different from that of Caucasian populations.
Aims: We aimed to study the characteristics of prospective whole-blood donors in the French West Indies in 2015 and to identify the factors influencing the recovery of hemoglobin levels after blood donation.
Methods: We included all individual blood donors who presented to donate deferred for anemia or not deferred, in Martinique (11,404 subjects) and in Guadeloupe (8,964 subjects). The characteristics of these prospective donors (sex, status, hemoglobin levels, number of previous donations, date of application) were available for the 2015 application and the previous donation. The analyses were stratified by sex and transfusion center.

Results: Mean hemoglobin levels for whole-blood donors was 12.92 g/dl for women and 14.72 g/dl for men in the French West Indies. Deferral for anemia was significantly more frequent in women (15.93%) than in men (3.43%). Hemoglobin levels recovered more rapidly in subjects with initially low levels and in women who had made many previous blood donations.
Summary/Conclusions: This study confirms certain sex-related physiological specificities of the recovery of pre-donation hemoglobin levels and supports the maintenance of this threshold hemoglobin levels for whole-blood donation in France, which is lower than in other European countries, but identical to that of New Zealand.

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ADVERSE REACTIONS IN BLOOD DONORS –OUR ONE YEAR EXPERIENCE

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Background: Whole blood donation is generally considered to be a safe procedure and voluntary blood donors normally tolerate blood donation very well. However, adverse reaction of variable severity may occur during or at the end of collection. Those adverse reactions can be divided into local reactions (problems with the venous access) and systemic reactions-which are divided into mild and severe.
Aims: To estimate the frequency and type of adverse reactions and to assess the practices which would help to avoid cause of unwanted reactions or help to minimize them.
Material and method: A retrospective study from our blood donating registers and registers for adverse reactions from January 2016 to December 2016. The donor population analyzed consisted of 1,902 donors -1539 male (84%), 363 female (16%).Voluntary were 1,830 (96%), family donors 72 (4%).First time donors were 297 (16%), regular donors were 1,605(84%).
Results: Only 12 adverse event were reported in that period in relation to 1902 donations, resulting in an overall adverse event rate of 0.6% that is an incidence of 1 in every 159 donation. 7 of them (4 female, 3 male-58% of all adverse events)-had local or mild systemic reactions (pain, swelling, agitation, nausea, presyncopal symptoms, pallor). 5 of them (42% of all adverse events- 4 female, 1 male)-had severe disorders, including vomiting, loss of consciousness, and convulsive syncope with loss of sphincter control. 10 of those blood donors with adverse reactions (or 83%) were first time donors. 8 of them were female (67%), 4 male (33%).
Summary/Conclusions: Only 0.6% of blood donations were complicated by adverse events and most of them were local or mild systemic. Adverse events were more frequent in female donors and first time donors. Our study confirmed the fact yet that the blood donation is a very safe procedure which could be made even more event-free by following certain friendly, reassuring and tactful practice. Donor care and the prospective reporting of donation related adverse events must become an integrative and self-evident part of hemovigilance registers.

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DESCRIPTION OF ADVERSE DONOR REACTIONS DURING AND IMMEDIATELY AFTER WHOLE BLOOD DONATION

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Background: Adverse donor reactions are a major cause of distress and the main reason for not opting to become repeat donors. Study of adverse donor reactions is important to identify the types of reactions and the contributory factors, and may help minimize or prevent adverse reactions in order to maintain a successful blood transfusion service with retention of blood donors.
Aims: The aim of the study was to assess the types and frequency of adverse donor effects and to identify predisposing factors in order to minimize or prevent unwanted adverse donor reactions.
Methods: A descriptive cross sectional single centre study was conducted on 1,000 conveniently selected whole blood donors, who participated at mobile blood donation campaigns conducted by the Teaching Hospital, Ragama. All adverse events occurring during or immediately after donation were noted using an interviewer administered questionnaire.
Results: Out of 1,000 blood donors, 264 (26.4%) were first timers while 736 (73.6%) were repeat donors; 809 (81%) were male and 191 (19%) were female. First time donors had a higher frequency of reactions (11%) than repeat donors (3.5%). The rates of reactions for males and females were 4.57% (37/809) and 9.24% (18/191) respectively. Overall 5.5% of donors experienced adverse donor reactions during or within 30 min of blood donation. The most frequent type of reaction was fainting or vasovagal reactions at 65% of total reactions while haematomas accounted for 21.6%. Vomiting (3.3%), convulsions (3.3%), chest pain (1.7%), numbness (1.7%), bruising (1.7%) and incontinence of urine (1.7%) comprised the rest. Prolonged loss of consciousness or arterial punctures was not present among the donors. Donors with a body weight of 50–60 kg, had a higher rate of adverse reactions (8.99%) than donors with a body weight of more than 60 kg (3.47%). Donors who had slept less than six hours had a higher rate of adverse reactions (19.51%) than those who had slept for six or more hours (4.9%). 25% of donors who fasted for six or more hours developed adverse reactions.. There were significant differences between blood donor adverse reactions and blood donors sex ($P = 0.008$), blood donation type ($P = 0.000$), age distribution ($P = 0.003$), weight of blood donors ($P = 0.000$), sleep duration ($P = 0.000$), and the fasting duration ($P = 0.000$).
Summary/Conclusions: The frequency of adverse effects related to whole blood donation is high. The most frequent reactions were, fainting or vasovagal attacks and haematomas. Adverse events are higher in females and in first time donors. Donors with low body weight, lack of sleep and long fasting periods prior to donation were more prone to develop adverse reactions.

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ADVERSE REACTIONS AMONG BLOOD DONORS IN KURDISTAN PROVINCE AND ITS EFFECT ON BLOOD DONOR RETURN RATES

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Background: Blood donation is widely considered to be safe with a low incidence of adverse reactions (ARs); however, ARs occasionally occur during or at the end of the blood collection. On the other hand, ARs can negatively affect donor retention.
Aims: The aim of this study was to assess the frequency of ARs in Kurdistan blood transfusion center, west of Iran, in 2015. We also determined the impact of ARs on donor return rates.
Methods: We performed a cohort study on whole blood donations, using data extracted from the blood service information. ARs were recorded using internationally agreed standard definitions. All donors were observed during and following donation for possible adverse events for 20 min. In addition, donors who had ARs were evaluated for return donation within 12 months and subsequent reactions.
Results: A total of 25,891 blood donors were enrolled. Of these, 20,476 (79.08%) were repeat donors and 5,415 (20.9%) were first time donors. Of the total number of donors, 170 (0.65%) experienced ARs; of these, 164 (96.4%) developed vasovagal reaction (VVR), 1 (0.5%) had arterial injury, and 5 (2.9%) developed hematoma. The mean age of female and male donors who experienced ARs was 31.4 and 30.73 years, respectively. In 87.05% (148/170), the donor was a man and in 58.8% (100/170) a repeat donation. Donors with hemoglobin less than 15 g/dl was significantly associated with VVR ($P = 0.004$). The return rate within 1 year was 18% (32/170) and all of them were repeat doors. In all, 78.1% (25/32) of donors who experienced ARs at the first donation had an uncomplicated second donation.
Summary/Conclusions: The incidence of reactions was low at our center. In addition, our results showed donation experience strongly influences on donor return and reduced donor return was seen following ARs.

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Abstract has been withdrawn.

Blood products

Blood processing, storage and release

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A METHOD FOR THE BIOTINYLATION OF RED BLOOD CELLS FOR CLINICAL RESEARCH THAT COMPLIES WITH GOOD MANUFACTURING PRACTICE REGULATION

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Background: Biotinylated RBC can be used to independently and accurately measure RBC survival and clearance. This method has several advantages over the standard ^{51}Cr method: (i) study subjects are not exposed to radiation; (ii) small blood volumes are required to analyze results, and (iii) multiple RBC populations can be measured simultaneously in the same individual. However, so far only experimental non-validated biotin-labeled RBC products have been transfused.
Aims: The goal of this study was to produce a standardized biotin-labeled RBC product in a fast, simple and sterile manner that can be used for clinical research and for the evaluation of new blood products.
Methods: Red Cell Concentrate (RCC) fractions were labeled with two different concentrations of biotin in a closed system (according to GMP), to ensure sterility of the labeled end product. Using a flow cytometric analysis, the reproducibility and robustness of the biotin labeling protocol was assessed, as well as the stability of the labeled (un-)irradiated end product. Additionally, RBC parameters such as phosphatidylserine exposure (PS), Na, K, free hemoglobin, ATP, pH and morphology were determined prior to and after biotin labeling to rule out effects of the labeling procedure on biological activity.
Results: Our data show that RCC can be labeled according to GMP, with two different biotin concentrations in a standardized manner, without affecting the biological activity.
Summary/Conclusions: An easy, rapid (<2 h), GMP qualified and robust method was developed to generate biotin-labeled RBC for clinical research.

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RESULTS OF THE IMPLEMENTATION OF AN AUTOMATED BLOOD PROCESSING SYSTEM AT TWO REGIONAL BLOOD TRANSFUSION CENTRES IN SWEDEN

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Background: Automation of blood component preparation represents a paradigm shift in whole blood processing. The Reveos automated blood processing system is able to simultaneously produce 4 leucoreduced plasma units, 4 leucoreduced red blood cell (RBC) concentrates and 4 interim platelet units in one single run.
This is the first report of the simultaneous implementation of Reveos ABCP system in two large Swedish university hospital blood banks, Lund (LU) and Gothenburg (GO), serving a total population of 2.3 million inhabitants.
Aims: The aim was to compare the quality of the Reveos processed blood components with the previously used semi-automated methods as well as to compare the outcome at the two different sites.
Methods: In LU 130 units were processed with the Reveos system and 48 units with previously used method (Fresenius Kabi blood bag (CQ32250) with subsequent platelet (PLT) production using the OrbiSac instrument). In GO 64 units were processed with Reveos and 64 units with the previously used method (Macopharma blood bag (1MALQT6281LE) with subsequent production of PLT using the TACSI instrument).
All RBC and PLT concentrates and plasma units were analysed for standard quality parameters.
The PLT activation marker CD62P was analysed in LU at day 1 and day 5 by flow cytometry on resting PLTs and PLTs stimulated with thrombin receptor agonist peptide (TRAP).
Results: RBC concentrates produced by Reveos in LU and GO showed almost an identical haemoglobin content (55.5 $\pm$ 4.5 and 54.0 $\pm$ 4.0 g/unit respectively) and RBC recovery (86.0 $\pm$ 3.0 and 84.5 $\pm$ 3.0%). Both parameters were significantly higher compared to the haemoglobin content (51.1 $\pm$ 4.1 and 46.6 $\pm$ 5.0 g/unit, $p < 0.05$) and RBC recovery (79.5 $\pm$ 2.0 and 76.5 $\pm$ 4.0%, $P < 0.05$) of units produced by the previously used methods.
Reveos plasma had a slightly lower volume compared with the previously used methods (246 $\pm$ 16 and 254 $\pm$ 22 vs 277 $\pm$ 16 and 274 $\pm$ 16 mL in LU and GO, respectively).
PLT concentrates from Reveos had almost the same amount of PLTs per unit in LU and GO (242 $\pm$ 45 and 252 $\pm$ 39*10^6/unit) and did not differ significantly from PLT concentrates from the semi-automated methods.
Reveos PLTs consumed less glucose and produced less lactate during storage and had significantly higher residual activation potential at the end of storage (88.0 $\pm$ 2.0 vs 74.0 $\pm$ 4.0%, $P < 0.05$) compared to the previously used methods.
The WBC concentration was $<1 \times 10^6$/l in all components.
Summary/Conclusions: The implementation of the Reveos automated blood processing system at two centres in Sweden resulted in blood components with almost identical quality characteristics. The Reveos system can ensure the production of blood components of unified quality, similar to or better than components obtained by semi-automated methods. Furthermore the Reveos system is easy to handle, robust and less labour intense and thus may enable a decentralized organization of the blood transfusion service in many countries.

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IN-VITRO EVALUATION OF THE EFFECTS OF LOW FREQUENCY (125KHZ) RADIO ENERGY ON RED CELL CONCENTRATES

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Background: The use of radiofrequency identification technology (RFID) in blood banking and transfusion medicine has the potential to advance patient safety, reduce costs and increase operational efficiency. As RFID becomes more widely deployed in healthcare the issue of safety has and is being evaluated with several previous studies reporting on the effects of high frequency (HF) radio energy (RE) at 13.56 mHz.
Aims: This study evaluates the safety of exposure, of various preparations of red cell concentrate (RCC), to extreme levels of low frequency (LF) RE at a wavelength of 125 kHz.
Methods: Ethical approval was obtained from the South African National Blood Service (SANBS) Human Research Ethics Committee.
Whole blood (WB) collection, RCC production and storage was carried out as per SANBS standard operating procedures. Using an approved limit testing protocol, units of RCC were aliquoted into test and control aliquots. Test aliquots were exposed to 220 μTelsa of RE over 24 h. Control aliquots were stored under similar conditions but without exposure. Exposure was carried out on Test aliquots at 5 days (YRBC) and 41 (ARBC) after WB collection. Test and control aliquots were sampled at 0, 7 and 24 h following the start of exposure and the samples were tested for evidence of cell degradation; red cell count, haemoglobin, haematocrit, plasma haemoglobin and potassium. In addition to 5 day testing, YRBC were stored and retested after 21 and 41 days.
Aliquot surface temperatures were logged every minute for the total exposure period and test and control temperatures compared to quantify relative test temperature increases.
Significant differences between test and control analytical results were evaluated using a two-tailed paired Student's *t*-Test.
Significant relative increases in test aliquot surface temperatures were evaluated by estimating the significance of positive slopes of normalised temperature differential plots for the uninterrupted 10–24 h period of exposure. Statistical significance was evaluated at the 0.05 significance level.
Protocol acceptance criteria were that haemolysis of both test and control groups be less than 1% after 24 h of exposure and that the maximum relative test temperature increase not exceed 1.5°C at any stage during the exposure period.
Results: After exposure YRBC haemolysis was 0.09%±0.03 and 0.09%±0.03 for test and control respectively. ARBC test haemolysis was 0.76%±0.57 and 0.83%±0.61 for the control after exposure. Haemolysis for 21-day YRBC test aliquots showed 0.22%±0.06 and control 0.18%±0.08 while after 42 days of storage the YRBC test and control haemolysis was 0.66%±0.24 and 0.59%±0.20 respectively.

No significant relative test aliquot surface temperature increases were observed for the YRBC ($y = 0.01x + 0.01$; p of $b = 0.09$) or ARBC ($y = 0.00x–2.86$; p of $b = 0.79$) exposure runs.
Analysis of markers for cellular degradation showed no significant differences between test and control aliquots.
Summary/Conclusions: The study data indicate no significant increase in cellular degradation or increased surface temperature in red cell concentrates following extreme exposure to low frequency radio energy at 125 kHz. The use of a RFID system utilising radio energy at 125 kHz is therefore unlikely to have a deleterious effect on RCC.

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DEVELOPMENT OF A MITOCHONDRIAL DNA MULTIPLEX REAL-TIME POLYMERASE CHAIN REACTION ASSAY FOR QUALITY CONTROL OF PATHOGEN INACTIVATION OF PLATELETS WITH UVC LIGHT

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Background: Several ultraviolet (UV) light-based pathogen inactivation (PI) technologies for platelet products have been developed or are under development. Upon implementation of PI technologies, quality control measures are required to ensure consistent efficacy of UV illumination process. Previous reports showed that amotosalen/UVA and riboflavin/UV-based PI technologies induce modifications of the platelet-derived mitochondrial DNA (mtDNA) that can be detected by polymerase chain reaction (PCR) inhibition assays.
Aims: To develop multiplex real-time PCR for detection of UVC-induced mtDNA modifications as a quality control for PI of platelets by the THERAFLEX UV-Platelets system.
Methods: Based on a binucleotide frequency analysis of the mtDNA genome, a multiplex real-time PCR assay was developed to simultaneously amplify short (143 bp)- and long (794 bp)-amplicons from a template DNA prepared using a platelet count adjusted DNA extraction method. Multiplex real-time PCR assay performance was evaluated on apheresis and buffy coat-derived UVC-treated and untreated plasma-reduced platelet concentrates (PCs) and challenged by using PCs with volumes, platelet counts and plasma contents at the upper and lower limits of the specifications defined for the THERAFLEX UV-Platelets system.
Results: PI of platelets using UVC light resulted in significant inhibition of PCR amplification of long-amplicon mtDNA targets relative to untreated products. Amplification of short-amplicon mtDNA targets was not affected by treatment. Evaluation of blinded platelet samples from routine-like production resulted in prediction of UVC treatment status with 100% accuracy.
Summary/Conclusions: A differential sized amplicon real-time PCR assay of mtDNA effectively documents nucleic acid damage induced by UVC illumination of platelets and could be used as an informative quality indicator of PI by the THERAFLEX UV-Platelets system.

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MAXIMISING PLATELET USAGE BY DELAYING REFRIGERATED STORAGE

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Background: Cold storage of platelets is being assessed as an alternative to conventional room temperature storage, particularly for the resuscitation of bleeding patients, due to their increased haemostatic potential. Cold storage also reduces the risk of bacterial growth and the accumulation of metabolic by-products. However, cold stored platelets are cleared more rapidly from the circulation and may not be appropriate for prophylactic transfusions. To maximise platelet inventory and minimise waste, platelets could be stored conventionally until near expiry (4 days) for prophylactic transfusions, then refrigerated to extend storage, for treatment of acute bleeding if required.
Aims: To determine whether delaying refrigerated storage of pooled platelets until near expiry is comparable to immediate refrigerated storage with respect to platelet quality and extending platelet shelf-life.
Methods: Two ABO-matched buffy coat-derived platelets (30% plasma/70% SSP+) were pooled and split to form matched pairs (n = 8 pairs). One unit was immediately stored at 2–6°C without agitation (day 1 post-collection; cold) while the second unit was stored at 20–24°C with constant agitation until day 4 then stored at 2–6°C thereafter (cold-delayed). All units were sampled on days 1, 4, 5, 7, 11, 14 and 21 post-collection and tested for *in vitro* quality. Data were analysed using a two-way repeated measures ANOVA.
Results: Over the entire storage period, cold and cold-delayed platelets maintained a similar platelet count ($P = 0.784$), expression of activation marker CD62P ($P = 0.199$) and release of sCD62P ($P = 0.260$). In addition, both phosphatidylserine exposure and microparticle release where similar between cold and cold-delayed platelets ($P = 0.220$ and $P = 0.943$ respectively). However, the increase in PAC-1 binding observed in cold platelets, under resting and ADP-stimulated conditions, was not observed in the cold-delayed platelets after refrigeration ($P < 0.001$). While pH was significantly higher in cold-delayed platelets ($P < 0.001$), other metabolic markers such as lactate ($P = 0.180$) and glucose concentration ($P = 0.214$) did not significantly differ during storage. Hypotonic shock response was maintained at a higher level in cold-delayed platelets ($P < 0.001$), whereas aggregation in response to ADP was lower compared to cold storage ($P = 0.021$).
Summary/Conclusions: The metabolic and activation profile of cold-delayed platelets was similar to cold stored platelets during a 21 day storage period, with the exception of PAC-1 binding. These data suggest that transferring platelets that are near expiry into refrigerated storage may be a viable option for maximising platelet inventories, by extending the shelf life of platelets beyond 5 days.

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MSCS CAN BE APPLIED IN RBCS STORAGE AS ONE KIND OF CELLULAR ADDITIVES

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Background: During storage in blood banks, RBCs undergo the mechanical and metabolic damage, which may lead to the diminished capacity to deliver oxygen. At high altitude region, the mentioned damage may get worse. Thus, more attentions should be paid to preserve RBCs when these components need transfer from plain to plateau region. Recently, we found that mesenchymal stromal cells (MSCs) could rescue anemia, and MSCs have been demonstrated in HSCs transplantation to reconstitute hematopoiesis *in vivo* by us.
Aims: We are trying to find out whether MSCs are helpful to RBCs in storage duration at high altitude.
Methods: We firstly checked the vitality of MSCs in CPDA-1at 4 ± 2°C for 14 days, and tested the quality of RBCs co-stored with MSCs, including the number of RBCs, Hct, Hb and the oxygen carrying capacity in preservation period. Moreover, we confirmed the effectiveness of RBCs co-stored with MSCs by transfusion in anemia models.
Results: We found MSCs were helpful to support RBCs to maintain biochemical parameters and kept RBCs function well on relieving anemia in an acute hemolytic murine model.
Summary/Conclusions: Our investigation developed a method to get a better storage of RBCs through adding MSCs, which may be applied in RBCs storage as one kind of cellular additives into preservation solution.

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VALIDATION OF PLATELET CONCENTRATE TRANSPORTATION IN A PNEUMATIC TUBE SYSTEM

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Background: Pneumatic tube systems (PTS) are used for transport of blood components, including platelet concentrates. However, limited research has been published on the impact of PTS transportation of platelets stored in additive solution. It remains unclear whether PTS transportation of platelet concentrates is advisable.
Aims: We set out to investigate whether PTS transportation alters platelet product quality compared to conventional transportation.
Methods: Identical pairs of platelet concentrates (n = 5) were transported using either PTS or manual transportation. The longest in-hospital transportation route was selected for a 2-way transportation of the product. One product was shipped 3 times by

PTS. Following transportation, the products were inspected for color change, aggregates, swirling, and foaming. Samples were drawn for measurement of pH_{22}, platelet concentration and for aggregation analysis by multiple electrode aggregometry.
Results: The two transportation modalities did not result in detectable variance in the product quality. No statistical significant differences among the groups could be identified. The average pH_{22} were identical, swirling was graduated as the highest level (+++) in all products, and no aggregates were identified. The platelet concentration varied by a maximum of 5%. Thrombin receptor-activating peptide-induced aggregation varied with a maximum of 23% (for the product shipped 3 times), for the remaining products a maximum variation of 10% was recorded. However, the aggregation results demonstrated no significant difference, and no trend of uniform alteration by transportation modality was detected.
Summary/Conclusions: In this study, limited by the small number of products transported, no impact of PTS on platelet product quality could be demonstrated. However, due to a considerable variation in the aggregation analysis, larger scaled studies would be needed to rule out a PTS induced impact on in vitro platelet aggregation.

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PERFORMANCE EVALUATION OF THE IN-LINE RED CELL FILTER LEUCOFLEX LCRD2 UP TO 72 H AFTER COLLECTION

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Background: In France, the current use of the In-line leucocyte reduction filter for Red Cell Concentrate (RCC) Leucoflex LCRD2 which is included in the quintuple top and bottom disposable NPT6280LA (MacoPharma) is limited to the filtration of RCC's at room temperature (18–24°C) up to 24 h after collection. New requirements of French Blood Agency (EFS) is to be able, to process whole blood (WB) beyond 24 h. Therefore we evaluated the efficiency of LCRD2 filter for up to 72 h storage at 2–6°C before filtration on RCC derived from Buffy-Coat (BC) method.
Aims: The objectives of this validation were to verify the conformity of filtered RCC filtered and to assess the performance of this method.
Methods: The study was conducted by 2 French blood centers (Besancon and Nantes). At least 204 RCC from WB units cooled to 4 ± 2°C up to 72 h were prepared and controlled. The 2 centers used their own validated processing and analytical methods. Whole blood units (target: 480 ml ± 10%) were collected with quintuple top and bottom disposables (NPT systems – MacoPharma) and stored after reception at 2–6°C until processing. After centrifugation, blood was automatically separated (Compomat G5 – Fresenius Kabi) with two modalities of BC extraction: N = 109 "normal" BC (Besancon = 50.7 ± 2.7 ml, Nantes= 50.9 ± 2.0 ml) or n = 95 "dry" BC (Besancon = 13.8 ± 1.1 ml, Nantes = 8.0 ± 1.3 ml). Filtrations were performed at 2–6°C. Filtration durations were recorded. A total of 204 RCC samples were analyzed the day of preparation for hemoglobin (Hb), hematocrit (Ht) and flow cytometry based residual WBC content. Hemolysis was measured on 43 RCCs after filtration and 21 days after donation. BCs were unsuitable for platelet preparation and plasma downgraded.
Results: 204 RCC were prepared. Time between collection and RCC processing was between 40 and 72 h. 1/204 RCC was out of specification for WBC count and the filter's investigation revealed clots. 1/204 RCC was out of specification for Hemoglobin (33.9 g/U) and Hematocrit (46.1%), the cause of this outlier result can be related to blood donation (436 ml WB and Hb donor equal at 11.4 g/dl). Two RCC prepared the same day in Besancon showed hemolysis after filtration and examination of the implicated filters revealed clots. The percentage hemolysis in RCCs increased during storage but no out of specification result was detected at Day 21. The average filtration duration was 1 h23 in Besançon and 1 h44 in Nantes.
Summary/Conclusions: The effectiveness of the in-line filter LCRD2 is unaffected by the storage of WB units at 2–6°C up to 72 h before filtration and all RCCs conformed to regulatory standards. The filtration duration allowed processing within routine processing conditions.

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IS DOUBLE-FILTERED LEUKOREDUCTION AN ALTERNATIVE TO IRRADIATION FOR THE PREVENTION OF TRANSFUSION-ASSOCIATED GRAFT-VS-HOST DISEASE?

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Background: Despite the broad application of filters for leukoreduction, transfusion-associated graft-vs-host disease (TA-GVHD) is still reported. Irradiation of blood products is required to prevent this, but installment of irradiators in all hospital blood banks can be a large burden. A recent survey in Korea showed that more than half of the blood banks participating in the survey do not provide irradiated blood, and 79.2% of the blood banks that do provide irradiated blood had irradiation done at an outside institution. Furthermore, referring irradiation to outside institutions can result in delays in the administration of blood products to patients, which can cause irradiation-induced hyperkalemia. With the accumulated advance in the quality of blood filters, we suggest the concept that filtered blood with near-zero residual leukocytes is sufficient to prevent TA-GVHD.
Aims: Under the hypothesis that complete leukocyte depletion does not induce TA-GVHD, we evaluated whether double filtration can accomplish zero residual leukocytes in red blood cell (RBC) products, as well as the effect of double filtration on the quality of blood products. This laboratory investigation can be a starting point for further clinical studies.
Methods: Thirty packed RBC products were filtered with RCM1 leukocyte filter (Haemonetics, Braintree, MA, USA), followed by 72 h of storage at 1–6°C, and application of a secondary filter (RC High Efficiency Leukocyte Removal Filter, Haemonetics). This process simulated the distribution of a blood product: an initial filtration by the blood supplier, a period of inventory at local clinics, and a secondary filtration at the bedside or hospital blood bank. Residual leukocytes were counted with LeucoCOUNT reagent (BD bioscience, San Jose, CA, USA) in a FACSCanto II (BD bioscience) flow cytometry device. The retrieved rate of RBCs after filtration was evaluated. Serum potassium and hemoglobin levels were measured before, directly after, and 35 days after filtration, and were used to evaluate the rate of RBC hemolysis. These parameters were compared to conventional RBC units, which had been filtered prior to refrigerated storage.
Results: Our study showed that single filtration can result in a 5-log reduction in filtering WBCs with an average loss of 11.9% RBCs, and that a second round of filtration showed no evidence of residual WBCs with an additional 1.5% loss of RBCs. Plasma hemoglobin and potassium can increase after the first filtration, and relatively small increase after the second round of filtration. Double-filtered RBC products showed little difference in RBC integrity compared to single filtration, and the increase in hemolysis post-filtration showed little difference, as well as the rate of hemolysis at day 35 was unchanged, suggesting that two rounds of filtration had no detrimental effects on RBCs in terms of spontaneous hemolysis. The most notable difference between single and double filtration was with respect to RBC recovery rate.
Summary/Conclusions: Our results demonstrated that modern, state-of-the-art blood filters can be used for blood products with zero residual leukocytes. Because there are circumstances in which irradiation is not possible, we suggest double filtration as an alternative approach to prevent TA-GVHD. Although broad adaptation of this process will need further investigation, we have demonstrated a relatively easy and adaptable method that can be applied instantly in situations where irradiation is not an option and the risk of TA-GVHD is high.

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IN-VITRO EFFECT OF LOW FREQUENCY (125 KHZ) RADIO ENERGY ON FROZEN AND THAWED PLASMA PRODUCTS

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Background: The use of radiofrequency identification technology (RFID) in blood banking and transfusion medicine has the potential to advance patient safety, reduce costs and increase operational efficiency. As RFID becomes more widely deployed in healthcare, the issue of the technology's safety has and is being evaluated with several previous studies reporting on the effects of high frequency radio energy (RE) at 13.56 mHz.
Aims: The current study evaluates the effects of exposure of various preparations of thawed and frozen plasma to extreme levels of low frequency (LF) RE at a wavelength of 125 kHz.

Methods: Ethical approval to perform the study was obtained from the South African National Blood Service (SANBS) Human Research Ethics Committee.
Whole blood (WB) collection, plasma production and storage was carried out according to SANBS standard operating procedures. Immediately prior to freezing, units of plasma were split into test and control aliquots. Using a limit testing protocol, three types of plasma preparations were tested; fresh frozen plasma (FP8), 24-h frozen plasma (FP24), frozen within 8 and 24 h of WB collection respectively and thawed frozen plasma (TFP), prepared as for FP8 and then thawed, at 37°C, after 72 h of frozen storage and subsequently stored at between 1°C and 6°C. After 72 h of storage, test aliquots of FP8 and FP24 (frozen) and thawed TFP were exposed to 220 μTelsa of RF power over 24 h at their respective storage temperatures. Control FP8, FP24 and TFP aliquots were stored under similar conditions but without RF exposure.
All plasma test and control aliquots were sampled at 0, 7 and 24 h following the start of exposure and were tested for fibrinogen, coagulation factors V, VIII, IX and XI, vWF antigen and proteins S and C.
Test and control aliquot surface temperatures were logged every minute for the total exposure period and compared to detect relative increases in test aliquot surface temperature.
Significant differences between test and control analytical results were evaluated using a two tailed paired Student's t-Test. Test aliquot surface temperature increases, relative to control aliquot surface temperature were evaluated by estimating the significance of positive slopes of normalised temperature differential plots for the uninterrupted 10–24 h period of exposure. Statistical significance was evaluated at the 0.05 significance level.
As per protocol, acceptance criteria were; test and control protein activity could not differ by more than 20% after exposure and the maximum relative test surface temperature increase was not to exceed 1.5°C at any stage during the exposure period.
Results: The mean difference between the test and control activity for any of the investigated proteins did not exceed 20% after exposure with no significant differences between test and control aliquot results detected. No significant relative test aliquot surface temperature increases were observed for the FP8 ($y = -0.02\times-2.58$; P of $b = 0.11$), and FP8 ($y = -0.03\times-0.56$; P of $b = 0.00$) and TFP ($y = -0.00\times-0.08$; P of $b = 0.51$) exposure runs.
Summary/Conclusions: The results show no evidence of significant protein degradation or product surface temperature increases in frozen and frozen thawed plasma when subjected to extreme low frequency radio energy exposure. The routine use of a RFID system which uses radio energy at 125 kHz is therefore unlikely to adversely affect these products.

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VALIDATION OF A NEW RESIDUAL CELLS COUNTING PLATFORM BY CYTOMETRY

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Background: White blood cells (WBC) in blood components are responsible for a number of well-known adverse effects in blood transfusion. The increasing use of WBC-reduced blood components has raised the need for a simple, stable and accurate routine method for counting low numbers of WBC.
Aims: The new BD FACSVia™ (BD Bioscience) flow cytometer was validated to replace a microscopic method using a Nageotte haemocytometer for the counting of residual white blood cells (rWBC) in Red Blood Cells Concentrates (RBCC) and Platelets Concentrates (PC) and for the counting of residual Red Blood Cells (rRBC), rWBC and platelets (PLT) in plasma, with the use of BD Leucocount™ and BD™ Plasma Count Kits.
Methods: BD FACSVia™ clinical software automatically adjusts key cytometer setup values to sustain optimal performance over time. Repeatability, intermediate precision and accuracy were determined by evaluating assays performed with both kits. Linearity was evaluated using the linear regression and the calculation of Pearson's coefficient (R^2). Cross-contamination over samples was tested. Assays were performed on calibrated high and low RBCC and PC controls. Bland-Altman graphs were applied to compare flow cytometric and microscopic counting methods. Limit of quantification (LOQ) was determined by counting events using 'blank' samples to define non-specific background.
Results: The coefficients of variation were less than 10% for high RBCC and PC controls and less than 20% for low RBCC and PC controls. The coefficient of variation in intermediate precision assays was inferior or equal to 10%. For the accuracy, the relative bias was inferior to 10% for PC high and RBCC high. The R^2 values for the linearity were 99.9% for RBCC and PC which were better than the acceptable limit of 98%. The cross-contamination analysis was negative. The LOQ (= 10×SD) was established and the result was 3 counted events, which corresponds to a concentration of 0.1 cell/μl considered as the LOQ for RBCC and PC components. For the BD™ Plasma Count kit, the repeatability results were less than 10% for the three blood components. Same results were found for the intermediate precision. The coefficients of correlation had a R^2 value of 99.9% for rWBC, 99.7% for rRBC and 100% for PLT. The cross-contamination analysis was negative, too.
Summary/Conclusions: The BD FACSVia™ cytometer was validated for the routine use to count rWBC in RBCC and PC with BD Leucocount™ kit and for the counting of rWBC, rRBC and PLT in plasma units with BD™ Plasma Count kit. This simple and reliable assay for the simultaneous cells determination can therefore replace time-consuming and laboratory intensive manual microscopic counting. Based on the linearity, accuracy, and intermediate precision, this technique allows the quantitative enumeration of the three cell populations of interest in the concentration range of their specification limits for blood components.

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POSITIVE EFFECT OF BICARBONATE ON IN VITRO RED BLOOD CELL QUALITY DURING STORAGE IN PAG3M

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Background: In Europe, red cell concentrates (RCC) are usually stored in SAGM (saline, adenine, glucose, mannitol). During storage, *in vitro* red cell quality declines, including lowered energy status and increased cell lysis. Recently, several additive solutions, designed to diminish the decline in *in vitro* quality during storage, were developed. These new solutions, including PAG3M and AS-7, are mainly developed to better maintain RBC 2,3-BPG levels and energy status during storage. Compared to AS-7, PAG3M maintains higher levels of 2,3-BPG, allowing better oxygen release, and higher energy status, which is necessary for function and survival of RBC *in vivo*. However, a recent study has shown that RBC storage in PAG3M has a negative effect on the deformability of RBCs. This effect was not seen with RBC storage in AS-7. An important difference between PAG3M and AS-7 is the presence of gluconate in PAG3M, while AS-7 contains bicarbonate.
Aims: To investigate the effect of various concentrations of bicarbonate on the *in vitro* quality of RBC during storage in PAG3M.
Methods: Four variants of PAG3M were tested: standard PAG3M; PAG3M + 52 mmol/l NaHCO3; PAG3M with 26 mmol/l gluconate replaced by 26 mmol/l NaHCO3; PAG3M with all gluconate replaced by 52 mmol/l NaHCO3. Overnight stored whole blood (n = 3) was leucocyte reduced and processed to plasma and packed RBCs. The packed RBCs were divided into 4 equal volumes and diluted with the PAG3M variants. During storage at 2–6°C for 35 days, RCC were weekly sampled and analysed for haematological, metabolic and rheological parameters.
Results: The internal pH of RBC stored in PAG3M variants with bicarbonate remained significantly higher than that of RBCs in PAG3M during the entire storage period.
ATP content at day 35 of storage showed no significant differences between the PAG3M variants, all values were around 5 μmol/g Hb. Haemolysis during storage was comparable for all PAG3M variants and was below 0.4% at the end of storage. At day 21 of storage, 2,3-BPG levels showed no significant differences between the PAG3M variants (21–25 μmol/g Hb). After 35 days of storage, the fraction of non-deformable cells, as measured with ARCA, was significantly lower for the PAG3M variants with reduced gluconate concentration. While with PAG3M, about 30% of the RBCs were non-deformable, this was reduced to 15–24% for the PAG3M variants.
Summary/Conclusions: Full or partial replacement of gluconate in PAG3M by bicarbonate has a positive effect on the deformability during storage without negatively affecting the positive effects of PAG3M on RBC metabolism (high ATP and 2,3-BPG levels).

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SYNTHESIS OF THE FIRST GMP GRADE BIOTIN 3-SULFO N-HYDROXYSUCCINIMIDE IS NOW AVAILABLE TO LABEL BLOOD CELLS IN-TENDED FOR HUMAN TRANSFUSION STUDIES

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Background: The recovery and survival measurements of autologous blood cells, in the circulation of healthy volunteers, is sometimes required by regulating agencies for acceptance of the new blood products. Such studies require that the transfused platelets or red blood cells (RBC) can be distinguished from the recipient's circulating cells. Currently, *in vivo* studies are mostly performed with radioactive reference tracers such as ^{111}In or ^{51}Cr. However due to regulatory obstacles, radiolabeling studies are only carried out in designated institutes. In many countries, however, radiolabeling of blood cells for studies in volunteers is not performed because of radiation exposure. Alternatively, biotin labeling method is used to track blood cells *in vivo*. A good correspondence between the survival of RBC labeled with either biotin or ^{51}Cr was demonstrated in a clinical trial (Mock, Transfus Med Rev, 2014). Biotin labeled RBC is safely transfused, even in neonates (Kuruvilla, Pediatr Res 2017). Concerning platelets, there is only one study in humans investigating the biotin method to estimate the recovery of stored platelets (Stohlawetz, Transfusion, 1999). These studies were all performed with a non GMP-biotin.
Aims: The aim of this study is to develop an alternative tracer (Biotin-Sulfo-NHS), synthetized in accordance with Q7 GMP Guidance for API and presenting several benefits: 1) non-radioactive compound, 2) safe, 3) lower cost, 4) limited device for tracking labeled cells by flow cytometry.
Methods: Synthesis of GMP-Biotin-Sulfo-NHS is performed by mixing *N*-hydroxysulfosuccinimide in anhydrous *N,N*-dimethylformamide and diclohexylcarbodiimide during 24 h under argon. The urea salts are filtered and washed with high grade water before lyophilization. The solid bulk of activated ester obtained was washed with methanol/ether. All chemical steps are performed according to the EudraLex guidelines: *The Rules Governing Medicinal Products in the European Union Volume 4; GMP; Medicinal Products for Human and Veterinary Use, Part II: Basic Requirements for API used as Starting Materials*. The multilabeling efficacy of RBC or platelets, with this GMP-Biotin is compared to a non GMP-biotin, by flow cytometry.
Results: Flow cytometry controls are performed with washed RBC, labeled with 2 densities of either GMP- or non GMP-biotin (Pierce, Rockford, IL), the mean fluorescence Intensities (MFI) obtained, overlaid. Biotin (µg/mL): 0 (MFI 0.4 vs. 0.4), 4 (MFI 11.6 vs. 9.5), 18 (MFI 44.1 vs. 42.3), 70 (MFI 174.2 vs. 178.8). Same results are obtained with washed platelets: 0 (MFI 0.4 vs. 0.4), 2 (MFI 3.7 vs. 3.7), 20 (MFI 34.5 vs. 32.6), 80 (MFI 232 vs. 232). No microparticles are detected excluding cell damage.
Summary/Conclusions: Measuring recovery and survival of blood cells is an important decisive factor when new blood products are developed. Therefore, the availability of this new tracer (Biotin-Sulfo-NHS, GMP grade) might facilitate the adoption of the "biotin labeling method" and the acceptance of clinical trials by the regulatory authorities, since it will increase the safety and will contribute to overcome the need for non-radioactive methods within the framework of human studies. This new tool will be helpful for blood banking practices, in hematology and transfusion medicine or in oncology-hematology.

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AUTOLOGOUS PERIPHERAL BLOOD STEM CELL COLLECTION IN CHILDREN WEIGHING LESS THAN 25 KG: COMPARISON OF TWO APHERESIS SYSTEMS

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Background: Autologous peripheral blood stem cells (PBSC) collection is becoming a routine procedure in hemato-oncology. However, in younger pediatric oncology patients with lower weights, it is still a challenge. Recently, the Spectra Optia (Terumo BCT, Tokyo, Japan) apheresis system was introduced.
Aims: The aim of this study was to compare the collection efficiency of the newly introduced Spectra Optia and the previously used CS3000 Plus (Fenwal Inc., Lake Zurich, USA) apheresis systems in children weighing less than 25 kg.
Methods: All pediatric oncology patients who underwent an autologous PBSC collection at the National Cancer Center in Korea, between January 2005 and December 2015, were retrospectively reviewed. Of these, 36 (18.4%) children weighed less than 25 kg. We evaluated the differences between the CS3000 Plus and Optia with respect to their apheresis procedures, parameters of apheresis, product, and collection.
Results: Eighty-eight collection procedures performed on 28 pediatric patients using the CS3000 Plus device were compared with 23 collections performed on 8 patients using the Optia device. The total processing volume (5,000 ml vs. 4,517 ml, $P = 0.008$), ratio of total plasma volume (TPV) to total blood volume (TBV) (4.6 vs. 3.6, $P = 0.001$), apheresis duration (190 min vs. 160 min, $P < 0.001$), and blood flow rate (32.5 ml/min vs. 40.0 ml/min, $P = 0.026$) were significantly different between the two apheresis systems, whereas there were no significant differences in the CD34 + cell collection efficiency (40.1 vs. 38.6, $P = 0.228$), platelet loss (53.3 vs. 46.3, $P = 0.079$), and product contamination RBC volume (5.1 vs. 4.9, $P = 0.405$) between both systems. No complication associated with citrate toxicity and other adverse effects were observed during the procedures involving both apheresis systems.
Summary/Conclusions: Optia is suitable for processing lower volumes and lower ratios of TPV to TBV. However, there is similar CD34 + collection efficiency and platelet loss compared with the CS3000 Plus in autologous PBSC collection. Optia demonstrated better collection efficiency, is comparatively safer, and yields more than the CS3000 Plus.

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ACOUSTOPHORETIC SEPARATION OF PLATELETS FROM WHOLE BLOOD: A RELEVANT AND PRACTICAL ALTERNATIVE TO CENTRIFUGATION

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Background: Shear-induced platelet activation is an unwanted side effect of the centrifugation-based procedure currently used in blood banks to prepare platelet concentrates. Transfusion of partly activated platelets could indeed increase the risk of adverse transfusion reactions.
Aims: Here we evaluated the effectiveness of an innovative acoustic-based fractionation device by carrying out a qualitative and functional *in vivo* analysis of isolated human platelets.
Methods: Whole blood was obtained from 14 donors and fractionated using an acoustic-based device. Platelet recovery and purity were determined by quantifying blood cell subpopulations in the microchannel outlet samples. Quality of isolated platelets was evaluated using the surface expression of two activation markers (P-selectin, PAC1) using flow cytometric methods while their procoagulant ability was investigated using *in vivo* experimentation. Platelets isolated using a soft-spin protocol, were used as inactivated control.
Results: Fractionation using the acoustic-based device led to a red blood cell clearance ratio from whole blood greater than 80% ($P < 0.001$) and a purity of platelets close to 91.0%. We did not find any difference in terms of quality and functionality of platelets from the same donors isolated using the acoustic device vs the soft-spin protocol.
Summary/Conclusions: This acoustic-based fractionation method led to excellent preservation of platelet quality and functionality providing a novel promising technique for whole blood fractionation in clinical settings.

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PLATELET TRANSFUSION ADVERSE EVENTS: PROTEOMICS STUDY

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Background: Blood platelets destined for transfusion release panoply of molecules during preparation and storage. The leukoreduction process made the transfusion safer but did not completely abolish the adverse events.

Aims: The rationale of this study is to identify potential proteins in Platelet Components (PCs) involved in serious adverse events (SAEs).
Methods: Pellets from leukodepleted PCs were sampled from 5 PCs implicated in adverse events and 5 PC matched controls.
We performed a Label-Free quantitative analysis using an LC-MS/MS method: LC system coupled to an Electrospray Q-Exactive quadrupole Orbitrap benchtop mass spectrometer. Subsequently, data were searched by SEQUEST through Proteome Discoverer 1.4. Raw LC-MS/MS data were imported in Progenesis QI 2.0 for peptide quantification and statistical comparison. Functional analysis was performed using Ingenuity Pathway Analysis software.
Results: 1,000 proteins were identified in our samples of which 423 were differentially expressed ($P < 0.05$, Fold Change>2) between the two studied groups. These 423 proteins revealed increased activation of platelets with degranulation and an intense modification of the structure of their cytoskeleton as well as an increase in their inflammatory functions in the event of an SAE.
The most enriched signaling pathways are: actin cytoskeleton signaling, the intrinsic pathway of mitochondrial apoptosis, integrin signaling, remodeling of epithelial adherens junctions, RhoA signaling, oxidative phosphorylation, signaling of the acute phase inflammatory response, and protein ubiquitination pathways.
Summary/Conclusions: The proteomic study of PC pellets induced by SAE may help to better understand the physiopathological aspect of SAE and thus may help to better prevent EIR in platelet-transfused patients.

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THE EFFECT OF LOW FREQUENCY (125 KHZ) RADIO ENERGY ON WHOLE BLOOD DERIVED PLATELET CONCENTRATES

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Background: The use of radiofrequency identification (RFID) in blood banking and transfusion medicine has the potential to advance patient safety, reduce costs and increase operational efficiency. As RFID becomes more widely deployed in healthcare, the issue of the technology's safety has and is being evaluated with several studies reporting on the effects of high frequency radio energy (RE) at 13.56 mHz.
Aims: This study evaluates the effect of extreme exposure of whole blood derived platelet concentrates (PLC) to low frequency (LF) RE at a wavelength of 125 kHz.
Methods: Ethical approval to perform the study was obtained from the South African National Blood Service (SANBS) Human Research Ethics Committee.
Whole blood (WB) collection, PLC production and storage was carried out according to SANBS standard operating procedures.
Using a limit testing protocol, units of PLC were split into test and control aliquots on day 2 after WB collection. On day 3, test aliquots were exposed to 220 μTelsa of LF RE power over a 24 h period. Control aliquots were stored under similar conditions but without exposure. During exposure PLC test and control aliquots were sampled at 0, 7 and 24 h, following the start of exposure, and the samples were tested for platelet count, PLC supernatant pH, lactate and aggregation response to 4 μg/ml collagen. After exposure test and control aliquots of PLC were stored and retested on day 6 after WB collection.
Test and control aliquot surface temperatures were logged every minute for the total exposure period and compared to quantify relative test aliquot surface temperature increases.
Differences between test and control analytical results were evaluated for statistical significance using a two-tailed paired Student's t-Test. Test aliquot surface temperature increases, relative to control, were evaluated by estimating the significance of positive slopes of normalised temperature differential plots for the uninterrupted 10–24 h period of exposure. Statistical significance was evaluated at the 0.05 significance level.
Protocol acceptance criteria were that test and control PLC supernatant mean pH should not be less than 6.2 after exposure and the maximum temperature increase of the test relative to the control would not to exceed 1.5°C at any stage during the exposure period.
Results: The mean test PLC supernatant pH was 6.55 ± 0.21 and the control 6.59 ± 0.21. No significant difference between the groups was observed after exposure. Similar results were found after 6 days of storage with the mean pH for test and control being 6.52 ± 0.21 and 6.49 ± 0.20 respectively.
No significant difference was observed between test and control analysis results after 24 h of exposure or after 5 days of storage.
No significant relative test aliquot surface temperature increase was observed for the PLC exposure run ($y = -0.02\times + 0.22$; p of b = 0.11).
Summary/Conclusions: From results of the results of this study, it appears that exposure to extreme levels of low frequency radio energy will not have a significant effect on PLC and therefore routine use of an RFID system, utilising radio energy at 125 kHz, would not have a deleterious effect on these products.

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IMPLEMENTING WHOLE BLOOD AUTOMATION AND PATHOGEN REDUCTION AT THE RED CROSS LUXEMBURG

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Background: The Red Cross Luxemburg (RCL) is the only blood establishment in Luxemburg. It collects annually about 570 thrombapheresis, 2,000 plasmapheresis and 20,000 whole blood donations. Recently, the RCL implemented the full-automated Reveos system that can process 4 whole blood units at a time, resulting each in one RCC (red cell concentrate), one plasma and one IPU unit (interim platelet unit). IPUs are then pooled to one PC dose (platelet concentrate) without any further centrifugation. Subsequently the Mirasol PRT system was introduced to replace gamma-irradiation and bacterial testing and increase protection against untested pathogens.
Aims: The purpose of this study is to report about the operational benefits brought to the routine blood component processing at RCL after the implementation of Reveos. Subsequent addition of the Mirasol PRT system to the routine is reported. Comparison of the current blood component processing with the previous semi-automated process will be reported.
Methods: Blood and blood components are collected and processed during 6 days in the week. In the processing lab 4 FTE (full-time employees) are responsible for processing all whole blood into components the whole year long, including vacation and occasional disease-related absences. Whole blood donations (475 ml) are processed with two protocols "fresh" (5 h post-collection) and "overnight" (up to 20 hs) into RCC, IPU and plasma. Plasma has to be frozen within 24 hs of collection. The Reveos software" T-IPU select" compiles information from the blood bank information system on ABO-Rhesus group and the Reveos software manager (RSM) to suggest combinations of 4–5 IPUs to create consistently PCs above minimal yield threshold and within the Mirasol treatment specifications, respecting time-frames of treatment.
Results: So far 34,138 whole blood donations have been processed with the Reveos system.. The average Hemoglobin content of the RCC is 54.9 g, plasma volume 261 ml and platelet yield of PC 3.46×10^{11}. Using three Reveos devices an average of 2 min per whole blood processing has been attained. The platelet pooling process is 10 min shorter than with the previous semi-automated system. Using "IPU select" a significantly narrower range of yield variation (37% of products between 3.4 and 3.8×10^{11}) was obtained with no products outside the Mirasol specifications. Hence, addition of the Mirasol PRT to the routine processing was realized without any augmentation of FTE or extension of working shifts. So far, 1,631 PC have been treated with the Mirasol PRT system and 1,629 released for transfusion with no reports of severe adverse reactions.
Summary/Conclusions: The implementation of the Reveos full automated system at the LRC increased its productivity through a simpler and swifter processing system. It enabled the blood center to implement Mirasol PRT and with that eliminating the need to add multiple additional safety measures (gamma-irradiation, bacterial screening) without increase in working hours or number of operators. The synergy of both technologies has been validated under routine conditions with so far favorable clinical results.

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Abstract has been withdrawn.

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DEVELOPMENT OF A RAPID IN VITRO ASSAY FOR PREDICTING SUITABLE LABORATORY ANIMAL SPECIES FOR STUDYING HUMAN HEMOGLOBIN INTERACTIONS WITH HAPTOGLOBIN IN VIVO

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Background: Human HbA is toxic when found in a cell free form and is rapidly captured in blood by haptoglobin. HbA and different variants of this tetrameric protein are under development for future safer hemoglobin based oxygen carriers. For the pre-clinical development of HBOCs and other HbA derived products, it is important to use laboratory animal species with haptoglobin interactions with HbA/HBOC that is similar as for human haptoglobin. Therefor, having a rapid *in vitro* assay to predict suitable laboratory animal species would be helpful.
Aims: To develop a cheap and rapid assay to predict animal species with haptoglobin that can interact with human HbA in a similar manner as human haptoglobin.
Furthermore, the assay should not require purification of haptoglobin proteins from the animal blood plasma and the animal haptoglobin interaction with HbA should be performed with both molecules in solution.
Methods: The assay was developed as a type of competitive ELISA. Human haptoglobin was first coated on a microtiter plate over night. A fixed amount of purified human adult hemoglobin (HbA) was pre-mixed with various dilutions of citrated plasma from different animals and incubated for 1 h in room temperature. After incubation, the premix was added to the microtiter plate wells and incubated for another hour in room temperature. Upon washing, bound HbA was detected with an antibody conjugate conjugated with horseradish peroxidase (HRP). Alternatively, bound human HbA was detected with anti-human HbA primary antibodies followed by secondary antibodies conjugated with HRP.
The resulting product was determined at 450 nm in a microtiter plate reader.
Results: The assay was first titrated with human plasma and a strong inhibition of HbA binding to coated human haptoglobin was observed at concentrations as low as <1% human plasma. The assay was further tested in pilot experiments using citrated plasma from different animals (mouse, chicken and catfish). All tested animal plasma samples displayed inhibition although not as potent as the human plasma.
Summary/Conclusions: The assay is promising as a novel method to screen suitable laboratory animal species in regards to the HbA interaction with haptoglobin. Since the plasma samples could be diluted about 100-fold and still demonstrate reliable results, the assay proved to be very cost-effective in terms of volume requirement for animal plasma. Upon further validation, this assay may be useful as a rapid screening method of laboratory animal species for studying human HbA interactions with haptoglobin *in vivo*.

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EFFECTS OF SUSPENDED RED BLOOD CELL SUPERNATANT ON HYPOXIA/REOXYGENATION INJURY IN H9C2 CELLS

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Background: During storage, suspended red blood cells (SRBCs) undergo changes in morphology, function, and metabolism, resulting in unique metabolomic and proteomic profiles for the supernatants of suspended red blood cells (SSRBCs). Transfusion of long-time preserved SSRBCs may lead to clinically adverse effects for recipients from a metabolomics standpoint. Although multiple studies found that longer storage of RBC units was associated with increased risks of infection, renal dysfunction, respiratory failure, multiple organ dysfunction syndrome, deep vein thrombosis, and mortality, especially in critically ill patients, this relationship has not been fully explored. To date, no study has focused specifically on measuring or modeling SSRBCs-related cytotoxicity after transfusion.
Aims: This study aimed to examine the in-vitro cardiotoxicity of SSRBCs on H9C2 cells subjected to hypoxia/reoxygenation(H/R) injury, which are sensitive to toxicants.
Methods: Five units of pre-stored leuko-reduced RBCs were donated by five healthy male donors (age: 25–31 years), and supernatant was isolated by centrifugation on days 0 and 35 of storage and stored at −80°C until use. To induce ischemia, cells were placed in an ischemic buffer in an incubator that was maintained with 0.1% O2, 5% CO2, and 95% N2 for 7 h. Subsequently, hypoxia-treated H9C2 cells were treated with serum-free DMEM or SSRBCs that had been stored for 0 or 35 days. Cells were reoxygenated and incubated in normal oxygen conditions for 2 h. We analyzed cell viability using the Cell Counting Kit-8 (CCK-8) assay, apoptosis using Acridine Orange/Ethidium Bromide (AO/EB) staining and an annexin V-FITC/PI assay, mitochondrial membrane potential (MMP) using a JC-1 staining assay, and ATP levels using the Enzy light ATP Assay kit.
Results: H/R-injured H9C2 cells showed significantly reduced cell viability, increased apoptosis, and decreased MMP compared to normally cultured cells. There were no significant differences between H9C2 cells treated with DMEM vs. SSRBCs stored for 0 or 35 days in terms of cell viability, apoptosis, MMP, or ATP level, nor were there any significant differences between the 0- and 35-days groups.
Summary/Conclusions: Supernatants from leuko-reduced RBC units stored for 0 and 35 days had no effects on H/R-injured H9C2 cells in terms of cell viability, apoptosis, MMP, or ATP level. This in-vitro result is consistent with recent clinical results that there is currently no evidence to support changing practices toward fresher RBC transfusions.

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COMPARISON OF THE RECOVERY RATES OF COAGULATION FACTOR VIII USING TWO DIFFERENT TECHNIQUES OF PRODUCING CRYOPRECIPITATE ANTIHEMOPHILIC FACTOR

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Background: Cryoprecipitate antihemophilic factor can be used to treat Hemophilia A caused by congenital deficiency of coagulation factor VIII, and used as adjuvant therapy of hemorrhagic disease caused by reduced coagulation factor. At present, main techniques for preparation of cryoprecipitate antihemophilic factor in blood components preparation department are siphonage and centrifugation.
Aims: To compare coagulation factor VIII in the products and in the source plasma and to calculate recovery rates by using the above-mentioned two different techniques to prepare the cryoprecipitate antihemophilic factor.
Methods: 200 bags of whole blood were divided into two groups, 100 bags each. Fresh frozen plasma (FFP) were prepared by using the same method. Cryoprecipitate antihemophilic factor was produced by using siphon and centrifugation techniques, respectively (Thawed at 4°C, separated with CompoMat G5, Fresenius-Kabi).
Results: The coagulation factor VIII in the cryoprecipitate antihemophilic factor prepared by using siphon technique and centrifugation technique were 113.63 ± 40.41 and 94.50 ± 21.85 IU/bag, respectively ($P < 0.05$); recovery rates were 62.72 ± 12.34 and 56.16 ± 10.01, respectively ($P < 0.05$) .
Summary/Conclusions: The centrifugation technique could be used for standardized of mass production cryoprecipitate antihemophilic factor since a blood component separation system with high degree of automation was used in centrifugal separation process. However, the source plasma used in the centrifugation technique was thawed slowly at 4°C for 18–24 h before centrifugation; the recovery rate of coagulation factor VIII in this technique was lower than that produced by siphon technique, due to the temperature rises in the source plasma during centrifugation process causing coagulation factor VIII to attenuate. The cryoprecipitate could be obtained within 1–2 h by water bath siphon technique, so the loss of coagulation factor VIII was reduced and a higher recovery rate was achieved. This method could be used in small batch and flexible production due to the low automation degree.

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IR THERMOMETER VALIDATION AS A BACKUP METHOD FOR THE MEASUREMENT OF BLOOD COMPONENT TEMPERATURE

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Background: The temperature of blood products during transport is routinely monitored by validated data loggers that must be set and activated beforehand. In the case of a data logger fault or human error, it is necessary to check the temperature using another method. Infrared (IR) thermometers are fast and easy to handle therefore we decided to perform their validation. According to the manufacturer, the accuracy of the purchased infrared thermometer is ± 1.8°C and repeatability ± 0.5°C.
Aims: Initially, we determined the most appropriate emissivity coefficient (ε) for blood bags material. The measurements were carried out for two different settings

with emissivity 0.85 and 0.95 (for red blood cells units in temperature range from 5 to 15°C) to establish which of them delivers better results. Later, we extended the validation with emissivity 0.95 for temperature range from 20 to 40°C (for whole blood units after collection) and for the range from 15 to 30°C (for platelet units).
Methods: We chose a method that allows a comparison of IR thermometer measurements with the core temperature of the blood component unit. NIST traceable temperature probe, which recorded the temperature every 15 s, was inserted into the blood bag containing outdated red blood cells or platelets. Blood components were cooled or heated and left min. one hour to equilibrate, put at room temperature and scanned by IR thermometer at regular intervals. Each measurement was repeated three times at a few seconds interval. For each scan, we recorded the exact time of measurement, which was later compared to the temperature reading of NIST thermometer at the same moment.
Results: The comparison of results (difference between NIST and IR, X ± 2sd) for emissivity 0.85 and 0.95 in a temperature range of 5–15°C showed better performance for emissivity 0.95, 1.9 ± 1.4°C for 0.85 (N = 51) and 0.41 ± 0.62°C for 0.95 (N = 261). The range of temperature from 20 to 40°C delivered the following results: 0.94 ± 0.78°C (N = 49) and for the range from 15 to 30°C, the results were 0.85 ± 0.6°C (N = 54). The repeatability of measurements was within ±0.5°C.
Summary/Conclusions: Comparison of surface temperature measured by an IR thermometer with the core temperature of blood components delivered acceptable results; therefore, a decision was made to use it as a backup method in the case of a data logger failure. Working with IR thermometer is easy and fast, although caution should be exercised to ensure dry blood bag surface, properly mixed component, right distance between device and the target, and to avoid scanning labels or surface over the air bubble.

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APPLICATION OF A PORTABLE MICROSCOPIC CELL COUNTER FOR THE COUNTING OF RESIDUAL LEUKOCYTES IN LEUKOREDUCED APHERESIS PLATELET CONCENTRATES IN A HOSPITAL BLOOD BANK

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Background: Reduction of white blood cells (WBCs) (leukoreduction) in blood products is essential for preventing many adverse transfusion reactions. As the level of leukoreduction is directly correlated with adverse transfusion reactions, an accurate and efficient enumeration method is required. The use of a portable microscopic cell counter in hospital blood banks can help efficient quality control of leukoreduction.
Aims: Herein, we evaluated the actual utility of this portable microscopic cell counter in a hospital blood bank with regard to the performance of multiple WBC filters and the overall time required to evaluate samples for remnant WBCs in blood products.
Methods: We acquired 100 specimens of apheresis platelets after transfusion. Apheresis platelets were filtered with filters provided by three different manufacturers. Remnant WBCs were measured by the use of Nageotte chamber, flow cytometry and ADAM-rWBC. The efficiency was calculated by measuring the time required for the analysis of one specimen ten times consecutively
Results: Compared with the manual counting, the cell counts from flow cytometry and the ADAM-rWBC were significantly higher, and the latter two methods were able to detect sporadic cases with residual WBC concentrations exceeding 1/μL. Evaluation of the time required by the primary testing personnel to evaluate residual WBCs in blood products showed that the ADAM-rWBC was the most time-efficient method. Flow cytometry and the ADAM-rWBC required similar analysis times, but as the ADAM-rWBC virtually required no time in post-analysis phase, it proved to be the most efficient tool in all three phases of analysis.
Summary/Conclusions: We have demonstrated that WBC filtered blood products can occasionally have unexpected levels of remnant WBCs after filtration. Although the levels of remnant WBCs after filtration are being tested within blood centers as part of their regular quality control programs, a simple and accurate method to enumerate remnant WBCs that is able to detect fluctuating levels in the hospital blood bank is required. Our observations suggest that the ADAM-rWBC and flow cytometry may be acceptable methods for this task and the portable microscopic cell counter offers the best time efficiency with similar effectiveness with the flow cytometric methods, suggesting that this method is best fit for hospital blood banks.

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AN EVALUATION OF THE FILTRATION PERFORMANCE OF IMUFLEX® WB-RP BLOOD BAG SYSTEM WITH INTEGRATED PLATELET REMOVING SEPACELL RZ-2000 FILTER

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Background: Leukoreduction or leukodepletion is defined as the removal of a significant part (on average 4 log reduction) of the white blood cells (WBCs) that are typically present in blood components. In pre-storage leukoreduction, the component is typically leukoreduced as part of processing and placed in storage. This form is most widely applied in Europe and in North America. The reduction in transfusion reactions has been demonstrated in a number of studies and has been a driving force behind the increase of leukoreduction of blood products. Transfusion reactions include cytomegalovirus transmission, febrile non-hemolytic transfusion reaction and HLA alloimmunization, which can lead to platelet refractoriness, whereby the platelet count response to platelet transfusions is significantly less than expected.
Aims: Here we present the evaluation of the IMUFLEX® WB-RP Blood Bag System (Terumo BCT) including the integrated Asahi Sepacell™ RZ-2000 high-efficiency leukocyte platelet removing whole blood (WB) filter.
Methods: Whole blood (WB) donations of 500 ml were collected in CPD in the primary bag and filtered (2 h after WB collection). Leukoreduction of WB donations (n = 20) was performed according to manufacturer's instructions. After filtration (platelet removing), the components were separated by centrifugation resulting in a red blood cell (RBC) component (stored in SAGM) and a plasma component. Quality parameters measured included: yield, hematocrit, hemoglobin and residual WBC and platelet content.
Results: RBC characteristics post-filtration and separation were as follows: weight (323 ± 35 g) hematocrit (54 ± 5%) hemoglobin (18 ± 2 g/dl); residual platelets (4–12 × 10^3/μL); residual WBCs (0.00–0.03 × 10^3/μL). The mean filtration time was 14 min and no filter blocks were observed. The average weight of the collected plasma was 261 ± 18 g.
Summary/Conclusions: The usability and performance of the IMUFLEX® WB-RP Blood Bag System with integrated Asahi Sepacell™ RZ-2000 platelet removing whole blood filter was evaluated. All quality parameters complied with Italian and European guidelines and requirements. In conclusion, the set was found to be a reliable and efficient collection and separation system that performs consistently.

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TEMPERATURE CONTROL AND TRACEABILITY OF THE FINAL LABELLING PROCESS OF THE FRESH FROZEN PLASMA BY THE KEEP-ICE PASSIVE SYSTEM

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Background: The final labelling process of the Fresh Frozen Plasma (FFP) is a very important element in the production process. During this stage it is difficult to document the temperature change suffered by the blood bag. The maintenance of the cold chain is a critical element for the holding time and the kind of use of FFP. The evidences required by the current standards of processing (traceability of the final labelling process and temperature certification during this process) have made necessary to find new technological solutions.
Aims: The aim of this work is to improve a method that absolves to these standards.
Methods: The KEEPICE system (M&G Int.'1, MB, Italia) for the management and the traceability of the final labelling process at a controlled temperature by conditioned plates has been experienced during the plasma processing cycle at the Blood Bank of Niguarda Hospital in Milan. The tested labelling batch has been traced to the following parameters: operator, cycle starting time, cycle ending time, identification unit code (CDM) by barcode reading, recording of the temperature of the sample blood bag surface through an infrared thermometer (OPTRIS GmbH) measurement made every 3 s and recording of the average value every 3 min.
Results: In February 2017 27 labelling PFC batches (613 units in total) were checked. The data analyzed were the following:
1). Average number of processed units per cycle: 22 + /−5;
2). Average labelling time: 18.3 + /−8 min;
3). Average starting temperature: −37.6 + /−8°C;
4). Average ending temperature: −34 + /−4.4°C.

The control procedure, in standard environmental working conditions, has pointed up the passage of the blood bag surface temperature from −30 °C to −2.7°C in 45 min.

Summary/Conclusions: Experimentation has shown that the KEEPICE instrument allows an effective control of the blood bag temperature during the labelling process maintaining a complete traceability of every passage. The labelling batch report allows for traceability of the processing performed, documents the compliance with prefixed standards without any alterations of the regular flow of activities.

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A NOVEL CENTRIFUGE AUTOBALANCING FEATURE INCREASES PRODUCTION EFFICIENCY WHILE MAINTAINING BLOOD COMPONENT QUALITY

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Background: To annually separate more than 80,000 bags of whole blood (WB) on the day of donation, the Unit of Blood Component Production, Karolinska University Hospital (Stockholm, Sweden), acquired five Macospin centrifuges (MacoPharma, Mouvaux, France). A 0.5 g maximum imbalance within centrifuge liner pairs is normally accepted, but the novel auto-balancing feature of Macospin allows 50 g imbalance. Eliminating manual balancing of centrifuge liners would save time and effort.

Aims: The aim of the study was to compare blood component separation using the auto-balancing system of MacoSpin to manually balancing the centrifuge liners.

Methods: For five centrifuges, 12 blood collection packs of BAT system NPT6280LE (MacoPharma) containing 450 ml ± 10% WB and 63 ml CPD were run at 3,130 × *g* for 11 min with (1) a maximum imbalance of 0.5 g (balanced) and (2) an imbalance ranging from 26 to 51 g within liner pairs (imbalanced). Additional overall rotor imbalance was created by placing the heavier liners in adjacent rotor buckets. Separation into RBCs, plasma and BC was done using MacoPress Smart Revo (MacoPharma) with a well-defined separation program. RBCs were weighed. BCs and unfiltered plasma were weighed and analyzed using a cell counter (Medonic CA620 Cellguard, Boule Medical AB, Spånga, Sweden). Hematocrit, RBC volume, plasma volume, platelet (PLT) count and PLT enrichment (BC-PLT count divided by donor PLT count) were determined for BCs. For plasma, the PLT count was assessed.

Results: For four centrifuges, no significant differences in BC-PLT count nor PLT enrichment were observed between balanced and imbalanced centrifuge cycles (CCs). For one centrifuge, the BC-PLT counts and PLT enrichment of the imbalanced cycle were significantly lower. For all centrifuges collectively, no statistically significant difference was detected between balanced and imbalanced CCs for either parameter.

For the other BC parameters analyzed, no significant differences between balanced and imbalanced CCs were observed, neither for individual centrifuges nor collectively.

Evaluating the plasma and RBC volumes revealed no significant difference for four centrifuges. One centrifuge showed a significantly higher plasma volume in combination with lower RBC volume for the imbalanced cycle, without affecting BC-PLT count. This resulted overall in a significantly higher plasma volume for imbalanced CCs compared to balanced CCs, but no significant difference for the RBC volume.

No PLTs were detectable in the plasma.

Summary/Conclusions: Lower BC PLT count for the imbalanced cycle of one centrifuge corresponds with lower PLT enrichment, suggesting it is likely related to this particular CC, e.g. inattentive bag folding technique.

Altogether, our results show that the auto-balancing feature of MacoSpin renders blood components as well separated as manually balanced centrifuge liners. This is supported by the absence of detectable platelets in the plasma for all CCs. The slightly increased plasma volume might even indicate better separation for imbalanced CCs.

Our conclusion is that with its auto-balancing feature, MacoSpin allows the time consuming manual balancing step of blood component production to be omitted, thereby increasing production efficiency. However, the imbalanced program needs to be carefully validated for each centrifuge.

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COMPLEX OF NANOPARAMETERS OF RBC MEMBRANES – BIOMARKERS FOR QUALITY ASSESSMENT OF PACKED RED CELLS BEFORE TRANSFUSION

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Background: Many studies have shown that after 20 days of storage the quality of packed red blood cells (PRBC) essentially decreases and questions arise about efficiency of transfusion. Severe post-transfusion complications may arise, especially in critically ill patients after massive transfusion. But uniform quality criteria for PRBC suitability for transfusion are not yet fully developed.

Aims: To suggest criteria for assessing of PRBC quality on base of study of membranes nanoparameters: the local membrane stiffness, nanoscale surface topology, local micro-morphological peculiarities of cells .

Methods: PRBC were preserved for 42 days under 4 °C. For study 1 ml of blood were withdrawn from each bag every 4 days. Local PRBC membranes stiffness was measured by atomic force spectroscopy. Force curves for native cells in liquid were measured. We used cantilevers SD-R150-T3L (Nanosensors, Switzerland), force constant 0.15–1.83 N/m, tip radius 150 nm.

Based on Hertz model we calculated local Young's modulus of cell membrane. To assess the possibility of membrane stiffness recovery *in vivo*, blood flow was simulated by thermostatic rotation of PRBC.

The nanosurface of PRBC membrane and morphological micro peculiarities were studied by atomic force microscopy in air. Cantilevers NSG01 (Nanosensors, Switzerland) were used, force constant 5 N/m, tip radius 10 nm. AFM images of cells ensemble 100 × 100 μm^2, individual cell 10 × 10 μm^2 and 500 × 500 nm^2 of membranes surface were obtained.

Results: In the first half of storage period (normal cells) the Young's modulus was 75 ± 12 kPa. On days 19–23 of PRBCs storage the local stiffness of the cell membranes sharply increased to 152 ± 36 kPa. Importantly normal cells during 12 h thermostatic rotation become softer by 10–18%. Wherein membrane stiffness after 23 days never recovered to normal values of Young's modulus. Moreover, in 20% they become stiffer.

Growth of membrane Young's modulus was correlated with the change of their nanostructure. Topological defects in the form of domains with a grain structure were appeared. Space period of nanostructures was 150–220 nm, height 10–30 nm. By day 33 domains were merged. Morphological changes of PRB were observed. Echinocytes and spheroechinocyte were dominated. On the 42nd day cells transformed into swollen ovalocytes. Their size reached 10–12 μm, and the nanoscale roughness leveled to 1–2 nm. After thermostated rotation of PRBC within 12 h, the cells did not restore any morphological or nanoscale parameters.

Summary/Conclusions: The set of modifications of membranes nanosurfaces was observed during prolonged PRBCs storage. Modification of nanosurface topology may lead to different post-transfusion pathology processes in blood vessels, in particular to activation of blood immune system directed on removing of cells with the damaged membranes from blood. The increased local stiffness of the membrane violates blood rheology and hemodynamics.

The evaluation of the complex of membranes nanoparameters should be essential procedure to assess the quality of PRBC membranes before cell transfusion.

The decision on the advisability of transfusion by proposed biomarkers can significantly reduce the risk of post-transfusion complications.

P-192

Abstract has been withdrawn.

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FREEZE-THAWING VS SONICATION PROCEDURE: THEIR EFFECTS ON GPIIB/IIIA AND GPIB PLATELET RECEPTORS DURING INFUSIBLE PLATELET MEMBRANE PREPARATION

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Background: Blood transfusion centers are under considerable pressure to produce platelet concentrates with a shelf life limited to 3–5 days. Many approaches have been investigated experimentally to produce new hemostatically active platelet products that are capable of long term storage. In this way during infusible platelet membrane (IPM) preparation as a platelet substitute two freeze-thawing and sonication methods were investigated

Aims: The purpose of this study was to evaluate effects of freeze-thawing and sonication methods during IPM preparation on the major aggregation and adhesion platelet receptors, CD41/CD61 and CD42b respectively.

Methods: Five random platelet concentrates were prepared from Tehran Blood Transfusion Center. These platelet units were pooled and centrifuged to remove WBC and RBC contamination and then were washed with sterile normal saline three times. Washed platelets was divided into two equal fractions. Freeze-thaw procedures was performed three times at −40 centigrade and room temperature respectively and the platelets of other fraction was disrupted by a sonication procedure and then flow cytometric analysis of CD41/CD61 and CD42b was performed.

Results: Flow cytometric analysis of CD41, CD61 and CD42b was found 89.08%, 80.89% and 16.00% for freeze-thawed sample and 55.67%, 68.92% and 17.64% for sonicated samples respectively.

Summary/Conclusions: Our results indicated that in spite of lower and nearly equal expression of CD42b for both methods, higher expression of CD41 and CD61 were observed for freeze-thaw procedure. This means that freeze-thaw procedure preserve better these major receptors in comparison with sonication procedure during IPM preparation process. Albeit, the findings of these investigations can improve IPM preparation as a platelet substitute and may affect patients' care in transfusion medicine in the future.

Blood components

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EFFICACY OF E-POLY-L-LYSINE AS AN ANTIBACTERIAL ADDITIVE FOR STORAGE OF HUMAN PLATELET CONCENTRATES

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Background: *Bacterial contamination* of platelet products is an *important* cause of *transfusion-associated morbidity and mortality*. E-Poly-L-lysine (e-PLL) is a polypeptide that has an approved antimicrobial food preservative.

Aims: This study aimed at evaluating the efficacy of e-PLL as an antibacterial additive for storage of human platelet concentrates (PCs).

Methods: 16 PCs units (mean volume 50 ml) freshly were collected in CPDA-1 anticoagulant from healthy blood donors who visited the blood bank of Tehran, Iran. All units were stored at 22–24°C on a flatbed agitator for 8 days. Staphylococcus epidermis, Pseudomonas aeruginosa, Escherichia coli and staphylococcus aureus (20 colony-forming units/ml) were inoculated into PCs. Thereafter, e-PLL was added to PCs at concentrations of 50, 100 and 200 μg/ml, respectively. In order to control bacterial inoculation of a PCs unit, aliquots of each with an e-PLL, and control PCs without e-PLL was collected on Days 1, 3, 5 and 8. The colony count was determined for PCs inoculated with each bacterium. Samples were analyzed for PCs parameters based on Na, k, pH, mean PLT volume (MPV) and PLT count.

Results: The minimum of e-PLL for inhibition of all bacteria in this study was 100 μg/ml in PCs after 8 days of incubation. There were no remarkable differences in the other variables, that is, PLT count, MPV, pH, plasma K and Na concentrations between the e-PLL–treated PCs and the controls.

Summary/Conclusions: e-PLL inhibited the growth of bacteria and did not considerably affect the quality of PCs.

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WHOLE BLOOD DERIVED SINGLE-DONOR PLATELET CONCENTRATES FROM DONORS WITH TYPE 2 DIABETES

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Background: Previously it was shown that donors could be classified as having platelets (PLT) with good, average or poor storage properties [Bontekoe, Transfusion, 2014]. A main difference between 'good' and 'poor' storage properties involved metabolic activity, resulting in a faster decline of pH during storage of 'poor' PLT concentrates (PC). This might be caused by a different functionality of the PLT mitochondria and there are indications that donors with a history of 'poor' PCs are more likely to have health issues, pointing towards Metabolic Syndrome and Type 2 diabetes (T2D).

Aims: Because of the strong rise of people with T2D in the Dutch population, the aim of this study was to characterize PLT from whole blood donors diagnosed for T2D, but accepted as donor.

Methods: Twelve whole blood donors with T2D, not using insulin, were selected and buffy coat (BC) and plasma were shipped to the laboratory. After overnight hold, a single-donor PC (sPC) was prepared. An equivalent number of sPC was prepared from age and sex matched control donors, derived from the same collection sessions. sPC were stored for 8 days at 22 ± 2°C in a 600 ml PVC-DEHP container (sub-optimal conditions) on a flatbed shaker and sampled on Day 1, 4 or 5 and 8 for determining the *in vitro* quality. The diabetic marker HbA1c was determined from red cells and cholesterol and triglyceride levels from plasma. From both groups 3 'good' (pH_{day8}>6.6) and 3 'poor' (pH_{day8}<6.3) storing sPC were selected and analysed in more detail.

Results: Donors were of age 57 ± 10 year and primarily men (75%). Donors with diabetes had a higher mean BMI (30.3 ± 4.6 vs.25.4 ± 3.4 kg/m^2), higher mean diastolic blood pressure before donation (92 ± 8 vs. 84 ± 7 mmHg) and higher HbA1c than controls. The sPC of both groups had the same volume (70 ± 5 vs. 72 ± 2 ml) and PLT content (71 ± 9 vs. 73 ± 11 × 10^9) but on Day 1 glucose concentration was higher in the diabetic group (20.5 ± 1.7 vs. 18.9 ± 1.4 mM, $P < 0.05$). On Day 8, the average *in vitro* quality was comparable in both groups (data not shown). When combining the selected 'good' and 'poor' storing PLT from both groups, a large difference in lactate production was observed (0.14 ± 0.04 vs. 0.36 ± 0.03 mmol/day/10^{11}PLT). The 'poor' PLT showed a faster decline of the mitochondrial membrane potential (as measured with JC-1) during storage than 'good' PLT. Remarkably, a significant difference in triglyceride levels was detected on Day 1 ('poor': 2.2 ± 0.7 vs. 'good': 1.1 ± 0.2, $P < 0.01$).

Summary/Conclusions: BC from donors with T2D who did not use insulin and fulfilled all donor criteria, were comparable with BC from age and sex matched controls, and seem suitable for preparation of PC. When selecting the 'good' and 'poor' storing PLT from the combined groups, the results of the previous study were confirmed, with significant differences in glycolysis rate and functionality of mitochondria. Metabolic Syndrome and T2D are still suspected as health issues involved in 'poor' storage of PLT because donors were of high mean age and because of the observed differences in triglyceride levels between 'good' and 'poor' stored PCs.

P-196

IN VITRO EVALUATION OF DEHT (BIS(2-ETHYLHEXYL) TEREPHTHALATE) PLASTICIZED PVC BLOOD BAGS FOR FRESH FROZEN PLASMA STORAGE AT 30 DAYS AND 1 YEAR

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Background: Di-(2-ethylhexyl) phthalate (DEHP) makes PVC film flexible and useful for blood products. During storage, DEHP can leach from the bag film into solution and be metabolized. Studies in rodents have suggested that exposure to DEHP may be associated with adverse health effects, albeit at high dosages. Both the FDA and EU allow the use of DEHP in medical devices. Nevertheless, attempts to find DEHP alternatives for blood bags have been difficult due to the RBC membrane-stabilizing effect of DEHP and its long performance history.

An alternative non-phthalate plasticizer, bis(2-ethylhexyl) terephthalate (DEHT) is structurally and functionally similar to DEHP, but distinct from a metabolic and toxicological standpoint. DEHT can undergo complete hydrolysis and has an excellent safety profile: it is not classified as a carcinogen, mutagen, reproductive toxicant or endocrine disruptor.

Aims: The in vitro study objective was to evaluate the quality of fresh frozen plasma (FFP) stored in DEHT plasticized containers compared to FFP stored in DEHP plasticized containers at 30 days and 1 year.

Methods: Thirty-six whole blood units were collected into CPD solution, leukoreduced, centrifuged, and separated into RBC's and plasma. ABO identical plasma units were pooled together in groups of three. The 12 pools included 5 group A, 6 group O and 1 group AB. Each plasma pool was weighed, mixed, sampled, divided into DEHP and DEHT pairs, and frozen at less than −20°C within 8 h of collection. In vitro plasma testing (PT, aPTT, Factor V, Factor VIII, Fibrinogen, Protein C, and Protein S) was done on Day 0, Day 30, and 1 Year of storage. DEHP and DEHT paired plasmas were thawed and tested at the same time.

Plasticizer concentrations were determined on Day 0, Day 30, and 1 Year of FFP storage. DEHP and DEHT and their monoesters were analyzed by liquid chromatography-mass spectrometry (LS-MS/MS). Internal standards were deuterated-DEHP, MEHP, DEHT and MEHT. Lower limits of quantification (LLOQ) were: DEHP = 2.9 ppm; MEHP = 0.3 ppm; DEHT = 0.9 ppm; and MEHT = 0.2 ppm.

Results: The mean and standard deviation (SD) for key plasma and plasticizer results are shown below.

Factor VIII (IU/dL) DEHP: Day 0, 118 ± 34; Day 30, 106 ± 28; 1 Year, 101 ± 26.
Factor VIII (IU/dL) DEHT: Day 0, 118 ± 34; Day 30, 104 ± 27; 1 Year, 98 ± 22.
Factor V (IU/dL) DEHP: Day 0, 97 ± 8; Day 30, 97 ± 7; 1 Year, 100 ± 10.
Factor V (IU/dL) DEHT: Day 0, 97 ± 8; Day 30, 95 ± 6; 1 Year, 97 ± 9.
DEHP content (ppm) in DEHP bag, 1 year, 8.6 ± 1.5.
DEHT content (ppm) in DEHT bag, 1 year, below LLOQ of 0.9.

There was no statistical difference in any plasma parameter between DEHP and DEHT bags at the same time period. Factor VIII retained greater than 80% of its initial value. The plasma stored in DEHT bags had an average plasticizer content 90% lower than that of the DEHP bags.

Summary/Conclusions: DEHT plasticized PVC containers provide similar FFP performance to that of DEHP plasticized bags. Since DEHT is less polar than DEHP its migration into plasma is also reduced. Based upon this data, DEHT is a potential replacement for DEHP in FFP storage bags.

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IMPROVED BACTERIAL DETECTION ALGORITHM SUPPORTS EXTENSION OF PLATELET STORAGE FROM 5 TO 7 DAYS AT CANADIAN BLOOD SERVICES

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Background: The shelf life for platelet concentrates at Canadian Blood Services is currently 5 days. A 5-day shelf life is mainly based on concerns about an increased risk of bacterial growth leading to septic transfusion reactions with longer platelet storage periods. Canadian Blood Services is proposing a bacterial testing protocol that would enhance bacterial screening and permit platelet storage for up to 7 days.

Aims: This spiking study was aimed at evaluating whether bacterial detection is improved when platelet screening with the BacT/ALERT system is performed at 36 h or 48 h post blood collection instead of 24 h as currently required. Enhancing bacterial detection with the addition of an anaerobic culture bottle and a 6-h post-sampling quarantine was also assessed.

Methods: Five bacteria, including four aerobic species (*Staphylococcus epidermidis, Staphylococcus aureus, Klebsiella pneumoniae* and *Serratia marcescens*) and one anaerobe (*Propionibacterium acnes*) were used in this study. Groups of four 7–8 day old buffy coat platelet pools (two for *P. acnes*) were tested for sterility with the BacT/ALERT system. The four platelet pools in each group were then inoculated at target bacterial loads of 0.003, 0.03, 0.3 and 3 colony forming units per milliliter (CFU)/ml), respectively. The target bacterial levels for *P. acnes* were 0.3 and 3 CFU/ml. Pools were then incubated under platelet storage conditions for 7 days. At times 24, 36, and 48 h post-inoculation, and every 24 h thereafter, 8-ml samples were injected into a BacT/ALERT aerobic and an anaerobic culture bottle. Samples were also taken at each testing time to determine bacterial loads by serial dilution and duplicate plating onto blood agar. Positive bottles were subcultured to confirm the identity of the inoculated species. Four independent repetitions were performed for each assay with each bacterial species. Statistical significance between times to detection in BacT/ALERT bottles was determined using the Wilcoxon signed-rank test. A P-value of <0.05 was considered statistically different.

Results: Positive culture results were obtained with fast growing aerobic bacteria (e.g., *K. pneumoniae*) at loads ≥0.004 CFU/ml when sampled at 24 h post-spiking. Importantly, platelet pools inoculated with *S. epidermidis* at levels 0.02–0.04 CFU/ml were mostly captured with sampling performed at 36 h and 48 h post-spiking. Platelet pools initially inoculated with approximately 0.002 CFU/ml of *S. epidermidis* were sometimes detected 3 days after spiking. Also notably, 24-h cultures of platelet concentrates initially inoculated with ≥0.04 CFU/ml of *K. pneumoniae* were captured within the 6 h post-sampling quarantine. Using anaerobic bottles allowed detection of *P. acnes* and showed a trend toward faster detection of *S. epidermidis* and *K. pneumoniae*. The rate of false positive anaerobic bottles observed was 0.9% (3/332).

Summary/Conclusions: A new platelet testing algorithm for bacterial contamination with delayed sampling from 24 h to ≥36 h, a 6 h post-sampling quarantine, and increased sampling volume, will improve platelet safety and supports the extension of platelet shelf-life to 7 days at Canadian Blood Services. Very low bacterial levels could still be missed however with platelet sampling at 36 or 48 h.

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DESTRUCTION OF PACKED RED BLOOD CELLS DURING LONG-TERM STORAGE DUE TO NANODEFECTS OF MEMBRANES

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Background: During long-term storage of packed red blood cells (PRBC) substantial changes of various biochemical parameters and cell morphology occur, because blood cells are in an artificial environment. The changes also affect intramembrane complexes of proteins. As a result, this may lead to degradation of blood rheology, reduction of gas transmission function after transfusion of such PRBC. The mechanism of all these PRBC disorders has not been thoroughly elucidated. It can be assumed that the basis of changes in the morphology of PRBC is a violation of membranes nanostructure of these cells.

Aims: of our investigation was to study microstructure changes of PRBC morphology and changes of cells membranes nanosurface during long-term storage by atomic force microscopy *(*AFM).

Methods: PRBC in hermetic blood bag (400 ml) with CPD preservative solution were obtained from 4 donors (Blood Transfusion Center, Moscow, Russian Federation). PRBC were stored at a temperature of 4°C during 42 days in accordance with WHO recommendations. 20 ml of blood were withdrawn from each bag without breaching of hermetic sealing every 7 days. AFM NTEGRA Prima (NT-MDT, RF) scanning of cells monolayers on glass was performed under the temperature of 19–20°C in liquid in contact mode, and in the air in semicontact mode without glutaraldehyde fixation.

Results: Alterations of RBC morphology were detected during of PRBC storage. On the 1–2nd days of storage 68 ± 8% of cells were discocytes. Number of echinocytes increased during the storage from 15 ± 4% till up to 52 ± 11% on the 16th day. By the end of the storage 78 ± 11% of cells became abnormal form.

Changing of the shape of cells was accompanied by the appearance of topological nanodefects on the membrane surface of PRBC.

By the 9–12th days topological defects in the form of domains appeared on the membrane surface. All domains consisted of discrete grains-like structures. The typical dimension of this topological defect was 120–200 nm. On the 16–23rd days the size of defects of membrane nanosurface increased up to 400–600 nm. Finally nanodefects were merged and morphology of the cells on the microscale was changed.

Summary/Conclusions: Our experiments show that nanodefects of membranes cause destruction of packed red blood cells during long-term storage. This is based on the process of damage of spectrin matrix during PRBC storage. This process is caused by biochemical phenomena due to oxidative processes during storage of PRBC.

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BLOOD BANK IMPLEMENTATION OF LOW TITER GROUP O WHOLE BLOOD FOR PREHOSPITAL USE

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Background: Military and civilian survival data indicates that early transfusion and use of plasma and platelets improves outcome. Haukeland University Hospital has approximately 10,400 active donors and supplies blood products to local hospitals, air ambulance services and military operations with a required shelf life of 7 days for air ambulance use and 14 days for military use.
Aims: 1) Implementation of a method for screening group O donors for low anti-A/B titers (IgM <250 and IgG <500). 2) Validation of the Imuflex WB-SP collection set featuring an inline platelet-sparing filter *(Terumo BCT)* to ensure that it meets guidelines of 450 ml volume (±10%), >43 g/unit hemoglobin, $<1.0 \times 10^6$/unit residual white blood cell count, <0.8% hemolysis and >80% platelet recovery. 3) Evaluation of Golden Hour containers with Crĕdo Duracube HD or ProMed cases *(Pelican BioThermal)* with Testo 174 T and Libero Ti1 loggers for continuous temperature monitoring to establish if the system can maintain 2–8°C for at least 3 days.
Methods: Anti-A/B titration of group O donors was performed by IAT. Briefly, 50 µl of 1:250 diluted plasma was added to a saline gel card for IgM determination and 25 µl of 1:500 diluted plasma to an anti-IgG gel card for IgG determination. 50 µl of 0.8% A1 and B cells were added and gel cards incubated for 15 min at room temperature (IgM) or 37°C (IgG). After 10-min centrifugation, the reactions were read manually. If negative, the donor was assigned a low titer attribute in the laboratory information system, which is used to search for and book donors for whole blood donation. 48 whole blood units were collected and samples taken for hemoglobin and residual white blood cell count. In addition, 30 units were tested for post-filtration platelet recovery and hemolysis on day 21. A container with two units of whole blood and a temperature logger was exposed to hot and cold environments over a 3-day period to evaluate temperature stability. Statistical process control of the whole blood product is performed by measuring collection volume, post-filtration hemoglobin, residual white blood cell count and hemolysis on day 21.
Results: 86% of group O donors screened over a 10-month period (n = 1,998) had low anti-A/B titers (IgM <250 and IgG <500). Evaluated whole blood units met the guidelines. Mean collection volume was 457 ± 11 ml. Leukoreduction took on average 38 ± 3 min, resulted in 11 ± 2% volume loss and had 84 ± 7% platelet recovery. Filter failure was not observed. Mean hemoglobin was 61 ± 4 g/unit, residual white blood cell count $0.03 \pm 0.04 \times 10^6$/unit and platelet count $77 \pm 15 \times 10^9$/unit. Mean hemolysis on day 21 was 0.2 ± 0.1%. The Golden Hour system maintained 2–6°C during the 3-day test period. Retrospective evaluation of temperature loggers from the air ambulance service showed no instances of temperatures below 2 or above 8°C.
Summary/Conclusions: Haukeland University Hospital has successfully established a whole blood donor program with approximately 1,800 donors currently screened as low titer. All evaluated units met the guidelines, and the storage and transport system ensures acceptable temperatures in a prehospital environment.

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A STUDY OF THE QUALITY OF RED CELLS FOLLOWING REJUVENATION, ADAPTED FOR CONCURRENT PROCESSING OF MULTIPLE UNITS IN A ROUTINE PRODUCTION ENVIRONMENT

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Background: 'Rejuvenation' of stored red cells has been shown to increase their ATP and 2,3-DPG to levels of freshly donated cells. The treatment involves adding rejuvesol® (red blood cell processing) Solution followed by warming with agitation, then cell washing. Wishing to validate conditions outwith those currently approved by FDA, we adapted this process to permit concurrent processing of multiple units, with simple measures to ensure process adherence. Also, the effects of some key variations to the process (e.g. starting volume of units, warming time or resuspension volumes) were evaluated.
Aims: To assess the effects of rejuvenation, and subsequent extended storage in SAGM, on red cell quality of units rejuvenated at different days of storage. To evaluate the effects of key process variables.
Methods: Six pools of 5 ABO/Rh-matched, leucocyte-depleted red cells in SAGM were made on the day after donation, then split to 4 units per pool, with samples taken as reference (D1; post-manufacture). On days 7, 21 and 28 one unit per pool was rejuvenated, by: (i) addition of 50 ml rejuvesol Solution followed by, (ii) warming, with agitation, for 60 min in a plasma thawer at 37°C, then, (iii) cell washing in SAGM, according to a 'manual' (centrifuge-based) process. These units were then stored to mimic 4 h chilled transport, then refrigerated for 96 h. Samples were taken pre- and post-rejuvenation and after 24, 48, 72 and 96 h of storage. Finally, one non-rejuvenated control unit from each pool was sampled at the end of shelf life (D35).
Additionally, low and high volume units were processed, sampling before and after rejuvenation.
During each rejuvenation process, simple data (time, unit weight, temperature) collection provided a sequence of measures ensuring process adherence. Samples were assessed for blood count and gases, supernatant qualities (microparticles, haemolysis, biochemistry) and cellular metabolites (ATP, 2,3-DPG).
Results: Rejuvenation successfully restored cellular metabolite levels at each of the chosen storage days – e.g. at D7, the earliest point of rejuvenation, rises in ATP (from 5.5 ± 0.2 to 7.2 ± 0.1 µmol/gHb) and 2,3-DPG (from 1.2 ± 0.2 to 14.9 ± 0.2 µmol/gHb; n = 6) back to fresh levels were observed on the processing day, the elevated levels being maintained to 72 h, each with an 11% drop at 96 h.
Cell supernatants showed the same pattern for all measures taken – e.g. potassium rose in untreated units from 1.8 ± 0.2(D1) to 15.6 ± 0.7(D7) to 45.2 ± 1.5 mM (D35; n = 6) but reduced to 1.6 ± 0.8 mM after rejuvenation on all three occasions, with a steady rise to 11.5 ± 1.1 mM after 96 h (n = 18).
To evaluate variations in rejuvesol Solution to red cell ratio, 50 ml rejuvesol Solution was applied to units (D22) that differed by 38% of red cell content (within specification). This restored ATP and 2,3-DPG levels with only 15% and 8% differences, respectively.
Summary/Conclusions: Rejuvenation elevates ATP and 2,3-DPG to fresh blood levels that are maintained upon extended storage and relatively robust to unit volume variations. Supernatant levels of haemoglobin, microparticles, potassium and lactate rose as expected in untreated units, were reduced by washing that follows rejuvenation, subsequently rising consistently over 96 h of storage, regardless of unit age.

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CRYOPRESERVED PLATELETS DEMONSTRATE A REDUCED RESPONSE TO COLLAGEN STIMULATION

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Background: Room-temperature stored (20–24°C) platelets require constant agitation and have a limited shelf-life of 5 days. Consequently, it is logistically challenging to supply remote and rural medical centres with platelet products. Cryopreservation of platelets is an attractive alternative to room-temperature storage, enabling an extended shelf-life of several years. Although cryopreserved platelets have been widely characterised under resting conditions, the effect of cryopreservation on their ability to undergo agonist-induced activation is yet to be fully explored. Collagen is a key modulator of platelet activation *in vivo* and was therefore assessed as an archetype for the activation capacity of platelets following cryopreservation.
Aims: To determine whether cryopreserved platelets were capable of responding to collagen stimulation *in vitro*.
Methods: Buffy-coat derived platelet concentrates (30% plasma/70% SSP+) were cryopreserved at −80°C using 5–6% dimethyl sulfoxide (DMSO). Once thawed, platelets were reconstituted in 30% plasma/70% SSP+. Paired platelets (n = 7) were sampled before freezing (RT) and after thawing (Cryo). Platelets were analysed under resting conditions and following collagen (10 µg/ml) stimulation. Platelet function was assessed using aggregometry. The mean fluorescence (MFI) of glycoproteins and activation markers was analysed by flow cytometry. Protein phosphorylation was investigated using Western blotting. Statistical comparisons were performed by ANOVA and a P-value <0.05 was considered significant.
Results: Cryopreserved platelets exhibited a significant reduction in collagen-induced aggregation (RT: 95 ± 7%; Cryo: 49 ± 4%). While the abundance of P-

selectin (CD62P) was higher in cryopreserved platelets (CD62P MFI RT: 27 ± 6; Cryo: 79 ± 10), no further increase was detected following collagen stimulation (MFI RT: 296 ± 90; Cryo: 72 ± 10). Similarly, the abundance of phosphatidylserine was increased following cryopreservation (Lactadherin MFI RT: 15 ± 2; Cryo: 1,636 ± 586) but remained comparable following collagen stimulation (MFI RT: 30 ± 13; Cryo: 1,928 ± 558). Although the abundance of activated integrin αIIbβ3 was reduced following cryopreservation (PAC-1 MFI RT: 211 ± 55, Cryo: 48 ± 15), collagen stimulation was able to activate integrin αIIbβ3 in cryopreserved platelets (MFI Cryo: 88 ± 13), although not to the same extent as room-temperature stored platelets (MFI RT: 888 ± 320). Proteins involved in mediating collagen signal transduction were assessed in order to understand the loss of function. The surface abundance of GPVI was significantly reduced in cryopreserved platelets (GPVI MFI RT: 2,169 ± 164; Cryo: 258 ± 48) as was the abundance and phosphorylation of Akt, Src, ERK, and p38 under basal and stimulated conditions.

Summary/Conclusions: Cryopreservation of platelets induces dramatic changes in the platelet phenotype and significantly impairs the response of platelets to collagen stimulation. This may be due to a loss of functional surface glycoproteins and consequent reduction in signal transduction. Although this study provides novel insight into the functional capacity of cryopreserved platelets, further efforts are required to delineate how the transfused platelet product achieves the reported haemostatic effects.

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STORAGE DURATION IS NOT A SOLO FACTOR IN THE DEFORMABILITY OF STORED RED BLOOD CELLS

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Background: Blood banking procedures are associated with damage to blood units, stored as packed red blood cell (PRBC), which can affect the transfusion outcome. Specifically, storage induces the impairment of the RBC deformability. Storage lesions to PRBC are well documented, but the extent of their clinical relevance is under considerable debate. PRBC storage duration is the sole criterion for PRBC inventory management, and blood units are supplied primarily according to first-in-first-out (FIFO) criterion, while the actual functionality, namely the effectiveness of the transfused RBC is ignored.

In a previous publication [Barshtein *et al., Expert Rev Cardiovasc Ther.* 5:743–52, 2007] we demonstrated a marked variability in the deformability of PRBC (stored 28 days). This could be the results of the storage procedure and/or due to initial inter-donor variability already in the freshly-collected RBC (Day 1).

Aims: To address this question, the present study was undertaken to comprehensively explore the deformability of freshly-collected blood and its alteration during routine cold storage, and to analyze the relative contribution of these factors to the actual deformability of stored PRBC.

Methods: *Study design:* RBC samples were obtained from 50 freshly-collected blood donations from healthy donors, 48 PRBC units (non-leukoreduced) stored in CPDA-1 for 35–37 days, and 55 PRBC units (non-leukoreduced) that were randomly used for transfusion. All samples were subjected to determination of cell deformability. In addition, the deformability of RBC from 14 leukoreduced PRBC units, stored in SAGM, were monitored from day of donation (Day 1) through storage for 35 days.

RBC deformability was determined, using our original computerized cell flow-properties analyzer (CFA), by cell elongation under shear stress, expressed by the elongation ratio, ER = ER = a/b, where a and b are the major and minor axes, respectively. E.g., ER = 1 reflects round RBC that are not deformed at the shear stress applied. The image analysis determines the ER for each individual cell and provides the deformability **distribution** in RBC population of 3,000–3,500 cells.

Results: The deformability of freshly-collected RBC exhibited marked variability already on Day 1. Routine cold-storage further induced a progressive reduction of PRBC deformability, and the variability between units increased with storage duration. At any storage duration, the actual PRBC deformability exhibited a clear dependence on the initial (Day 1) value.

Summary/Conclusions: At any storage duration, there is great variability in PRBC deformability, which is determined primarily by initial, Day-1, level, and is further reduced, although moderately, with storage duration, implying that the effect of storage duration is only one, and seemingly not the important one, of the determinants of the PRBC deformability. In a recent study, we have demonstrated that regardless of storage duration, the deformability of transfused PRBC is a potent effector of the transfusion outcome, as expressed by the transfusion-induced change in the recipient's blood flow and hemoglobin increment. This implies that the FIFO criterion is not sufficient for assessing the transfusion effectiveness. The determination of PRBC deformability, independent of storage duration, is essential for the assessment of the potential effect of transfused PRBC on the transfusion outcome.

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SINGLE-DONOR PLATELET CONCENTRATES FROM WHOLE BLOOD DONATIONS WITH COLLECTION DRAWING TIMES BETWEEN 12 AND 15 MIN

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Background: The collection time of whole blood is, according to European Guidelines, limited to 15 min. In addition, donations with collection times between 12 and 15 min should not be used for preparation of platelet (PLT) concentrates (PC) because of the chance of too much activation of PLT. It seems justified to re-evaluate the quality of PLT from these donations because new generations collection systems and mixers were introduced, including a more efficient needle.

Aims: To investigate the *in vitro* quality of PC prepared from 12 to 15 min buffy coats (BC) with the aim to prevent unnecessary discarding of BC and to simplify the total blood bank process.

Methods: Single-donor PC (sPC, n = 6) were prepared from one 12–15 min BC and 60 ml of plasma in a 600 ml PVC-DEHP container. As a reference, sPC from donations with collection times of <12 min were prepared (n = 5). In addition, PC were prepared from 5 BC, of which at least 4 BC were from 12 to 15 min donations (n = 5). After pooling of the BC, 300 ml of PAS-E was added and a standard pooling set with a PVC-BTHC storage container was used for storage of PC. All PC were stored for 8 days at 22 ± 2°C and sampled at regular intervals for determination of the *in vitro* quality. Aggregation tests were performed with Chronolog (ADP or collagen) and Multiplate (arachidonic acid) aggregometers. Thromboelastography (TEG), using kaolin as an activator, was applied for assessment of the overall clotting capacity. Values are expressed as mean±SD. A non-paired t-test or a Mann-Whitney U test was applied for statistical analyses of normal or non-normal distributed data respectively.

Results: Volume (67 ± 5 vs. 66 ± 16 ml) and platelet content (74 ± 11 vs. 71 ± 15 × 10^9) were similar in both groups. At the end of storage, both groups showed comparable *in vitro* quality (Day 8, pH(37°C): 6.84 ± 0.16 vs. 6.83 ± 0.17, other data not shown). No differences in aggregation response after stimulation with arachidonic acid, ADP or collagen were measured. TEG parameters in both groups were also comparable. The five-donor PC fulfilled all requirements of European Guidelines, aside from occurrence of small aggregates at Day 6 and/or 8 in 2/5 PC (possibly because sometimes AB0 incompatibility was accepted). On Day 8, PLT showed low CD62P expression (17.1 ± 1.8%) and phosphatidylserine exposure (Annexin V binding, 8.9 ± 1.9%). Hypotonic shock response of platelets was comparable with historical data.

Summary/Conclusions: Single-PC in plasma as well as five-donor PC in PAS-E, prepared from 12 to 15 min whole blood donations had a normal composition and showed good *in vitro* quality during 8 day storage. To substantiate that the exclusion of 12–15 min donations for PC preparation could be stopped, further studies will be performed.

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COMPARISON OF COAGULATION FACTORS IN FROZEN PLASMA AND CRYOPRECIPITATE PREPARED FROM WHOLE BLOOD HELD AT 8 VS. 24 H AT ROOM TEMPERATURE

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Background: We switched from platelet rich plasma to buffy coat (BC) method for processing of platelets from whole blood (WB) in 2012 due to its operational benefits, but continued to process plasma as fresh frozen plasma (FFP). This posed a logistical challenge because the BC method involved holding WB overnight at room temperature before separation into components, while FFP needed to be frozen within 8 h of collection. Studies have shown comparable activities of coagulation factors between FFP and frozen plasma prepared from WB stored at room temperature up to 24 h (RTFP24).

Aims: We conducted a validation study to compare clinically relevant coagulation factors and quality control (QC) indicators at the time of thaw between FFP and RTFP24, as well as between cryoprecipitate prepared from FFP and RTFP24.
Methods: 20 units (U) each of RTFP24 and FFP (blood groups O: 8U, A: 5U, B: 5U, AB: 2U) were processed and frozen from CPD-stored WB. These were thawed ≥3 weeks later and measured for FII, FV, FVII-XI, FXIII (quantitative), fibrinogen, vWF:Ag, protein C, protein S (functional) and antithrombin IIII at the time of thaw. 10 single cryoprecipitate units were each prepared from FFP and RTFP24 (blood groups O: 5U, A: 2U, B 2U, AB: 1U). These were measured for FXIII (quantitative), FVIII and fibrinogen at the time of thaw. The data was analysed for differences between RTFP24 and FFP using non-parametric tests.
Results: FFP was thawed later than RTFP24 after freezing (median (range): 112 (21–133) vs 98 (28–105) days, $P < 0.001$). Compared to FFP, RTFP24 was not significantly different in clotting factors, vWF:Ag and natural anticoagulants except for lower protein S (median (range): 99 (70–116) vs 76 (57–102) IU/dL, $P < 0.01$) and higher FXIII (median (range): 90 (67–129) vs 110 (85–151) IU/dL, $P = 0.03$) at the time of thaw. These differences were unlikely to have significant clinical impact. FV showed a tendency to be lower in RTFP24 than FFP (median (range): 98 (68–164) vs 109 (92–135) IU/dL, $P = 0.052$) but was likely of no clinical significance since all RTFP24 units had FV levels above the lower limit of the reference range. The proportion of RTFP24 units meeting our plasma QC requirement of FVIII >70 IU/dl was similar to FFP (75% vs 85%, $P = 0.7$). There was no significant differences in fibrinogen, FXIII and FVIII content ($P > 0.6$) between cryoprecipitate derived from RTFP24 and FFP. All RTFP24- and FFP-derived cryoprecipitate met our QC requirement of ≥150 mg/unit of fibrinogen (range 171–425 mg/unit for RTFP24-derived cryoprecipitate; range 214–468 mg/unit for FFP-derived cryoprecipitate). 20% of RTFP24-derived cryoprecipitate (all group O) did not meet our QC requirement of ≥80 IU/unit of FVIII unlike all FFP-derived cryoprecipitate, although the difference was not statistically significant ($P = 0.47$). Moreover, this would have no clinical impact since we do not use cryoprecipitate to replace FVIII.
Summary/Conclusions: It is acceptable for us to switch from FFP to RTFP24 for the processing of plasma and cryoprecipitate since they have comparable coagulation factors at the time of thaw.

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Abstract has been withdrawn.

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COMPONENT PORTFOLIO FOR LOW LEVEL ANTI-T FRESH FROZEN PLASMA (FFP) FOR PAEDIATRIC PATIENT WITH NECROTIZING ENTEROCOLITIS (NEC)

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Background: Naturally occurring Anti-T was present in the plasma of all individuals. It is formed after the exposure to T-antigens present on many gram negative bacteria and vaccines. However in paediatric patients with Necrotizing enterocolitis (NEC) and Atypical Haemolytic Uraemic Syndrome (aHUS), neuraminidase remove the sialic acid residues on red cells and expose T antigens. Transfusion of blood product with high level of Anti-T may result of severe haemolysis. NHS Blood and Transplant (NHSBT), UK has been supplying low anti-T level paediatric fresh frozen plasma (FFP) for clinical use. A level of Anti-T titre of 4 or below had been selected with no conclusive supportive evident. In order to establish the proper component portfolio for the level of Anti-T in adult male donors.
Aims: A study was carried out to identity the level of anti-T level in selected group AB male donors in England.
Methods: A total of 534 plasma samples (group AB, antibody screen negative) male donor were tested for the level of Anti-T antibody by saline agglutination technique. Neuraminidase enzyme treated group O donor cells was tested in parallel with monoclonal Anti-T (OSK23, IgM) by doubling serial titration. The relationship of age, D status and area of residence was analysed from the information obtained by PULSE computer system for NHSBT.
Results: A total of 534 group AB male donor's samples were tested, the age of these donor ranged from 24 to 77 (mean 55.05) year old. Out of 413 donors were D+ (77.3%) which were slight lower than the 85% of normal Caucasian population
Anti-T titre level was as followings:
Titre 2: 38 (6.35%) Mean titration score: 13.53
Titre 4: 88 (16.4%) Mean titration score: 21.69
Titre 8: 99 (18.5%) Mean titration score: 31.34
Titre 16: 99 (18.5%) Mean titration score: 45.13
Titre 32: 121 (22.7%) Mean titration score: 57.08
Titre 64: 35 (12.0%) Mean titration score: 70.10
Titre 128: 19 (3.6%) Mean titration score: 79.90
Titre 256: 68 (12.7%) Mean titration score: 87.17
Titre 512: 6 (1.1%) Mean titration score: 97.16
The normal range for the "Titre" was from 2 to 256 using EP Evaluator and "Score" was from 13 to 91. There were six donors with high level of Anti-T (titre 512) and the average age 49 (range 38 to 65, mean 51.5) years old.
Summary/Conclusions: There was no significant different for the level of Anti-T in relationship with age and D status. Unfortunately, the product required must be group AB, so it was not a true representative of the normal range of male Anti-T level. Also the donors selected must be CMV negative. Currently NHSBT uses titre 4 or below as the criteria for such high specified fresh frozen plasma (FFP) for such paediatric patient. From this study, it is possible to select very low level of Anti-T (titre 2, n = 38, 7.1%) and (titre 4, n = 88, 16.5%). Once the products were selected during screening and confirmation of low level of Anti-T. The selected FFP were methylene blue treated and retrospective tested for HEV.

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COMPARISON OF PLATELETS IN ADDITIVE SOLUTION POOLED AND STORED IN THREE DIFFERENT POOLING SYSTEM

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Background: The *in vitro* quality of platelet concentrates (PCs) and the *in vivo* effectiveness of platelets (PLTs) depend on various factors like leukoreduction, storage temperature, gas exchange capabilities of the PLT storage container, PLT concentration in the storage container and duration of storage. Significant number of these factors depend on the characteristics of pooling systems components such as leukoreduction filters and PLT storage containers.
Aims: Evaluation in vitro quality of BC-derived platelets in additive solution prepared and stored in three different pooling system for 5 days: Haemonetics (ATSBC 1EPSB) and Fresenius (PT52600) with autostop filters and Therumo (TF*FP0610M1) with non-autostop filter.
Methods: Donor differences were avoided by applying pool-and-split design. Twelve ABO and RhD-matched BCs were pooled, mixed and then equally divided into the three pooling containers of different PLT pooling systems, each of which was supplemented with one unit of SSP+ additive solution (250 ml). After soft-spin centrifugation PLT-rich supernatant was pressed using a blood component separator (Optipress II, Fenwal). First phase of separation (until RBCs reach top optic sensor) was done automatically and second phase manually. Manual pressing was stopped at the moment when the filter was completely filled with RBCs (in case of autostop filters) or at the moment when the RBCs left the outlet of the filter (in case of non-autostop filter). Ten triplicates of PCs were prepared and stored in Haemonetics, Fresenius and Terumo system for 5 days. On days 1 and 5 of storage, samples were tested for platelet concentration, mean platelet volume (MPV), pO_2, pCO_2, glucose, lactate, bicarbonate, pH, CD62P.
Results: All platelet concentrates met national and Council of Europe guidelines for platelet products. There were no statistically significant differences between Haemonetics, Fresenius and Terumo systems regards the number of residual leucocytes, MPV, bicarbonate, pO2 and CD62P. PCs in Terumo system showed highest PLT recovery of 79.5% from BC pool compared to Haemonetics 74.7% and Fresenius 73.4% ($P < 0.05$). PCs in Terumo on Day 5 had highest PLT count and concentration ($P < 0.05$). PCs in Terumo had lowest pH ($P < 0.05$) but still well above the limit (>6.4) on Day 5. pCO2 decreased during storage in all pooling systems but PCs in Fresenius had lowest pCO2 on Day 5 ($P < 0.05$). Glucose decreased and lactate increased during storage for PCs in all pooling systems. PCs in Terumo system had higher glucose metabolism ($P < 0.05$) than PCs in Fresenius and Haemonetics system

as seen by a larger reduction in the glucose concentration and higher formation of lactate than PCs in Haemonetics and Fresenius system.
Summary/Conclusions: Although PCs begun as identical BC pools, they were processed using different pooling systems to evaluate effect of different leukoreduction filters and storage containers on quality of PCs.
PCs in Terumo system had highest recovery and platelet concentration at the end of storage, although the pH and glucose level were lower than for other two pooling systems which suggest higher PLT metabolism. This may be result of higher number of PLT in PCs. However, pH level was within acceptable range during 5 days storage period for PCs in Terumo.

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FIVE YEARS FOLLOW UP OF BLOOD PRODUCTS QUALITY CONTROL AT THE ETABLISSEMENT FRANÇAIS DU SANG, THE FRENCH TRANSFUSION PUBLIC SERVICE

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Background: Strategies and technological choices retained by blood transfusion centers for collection and preparation of labile blood products can have a significant impact on quality and characteristics of final products. Implementation of a quality control policy, based on a regular sampling plan, validated analytical methods and standardized data collection can be a powerful tool to reflect product specifications for each process line and assess the impact of technological strategies on the range of product provision.
Aims: To use regular Quality Control (QC) results to assess the impact of different technical solutions and processes on the quality and conformity of blood products to regulatory requirements.
Methods: The Etablissement Français du Sang (EFS) is the unique national blood transfusion operator in France. All transfusion activities, from collection to issuing to patient are implemented by regional transfusion establishments. For the last five year EFS provided 12 M units of RCC, 1.5 M units of platelet concentrates and 2 M units of FFP. Fifteen quality control laboratories ensure conformity and quality of the blood products delivered by 15 production centers. Results feed a national QC database on a continuous flow basis, thus allowing monthly trend analysis and conformity confidence on a nationwide scale. All available results are associated to a collection and production process.
Results: For the last 5 years (2012–2016), we have collected complete QC results from randomly selected 75,000 RCC, 35,000 Apheresis Platelet Concentrates (APC), 25,000 Pooled Buffy-coat derived platelet concentrates (BcPC) and 70,000 FFP. Results for RCCs relate to two main whole blood derived process (Whole blood filtration or BAT) and one apheresis process. Results for APCs relate to three main apheresis technologies, AMICUS (Fresenius), TRIMA (Terumo BCT) and MCS+ (Haemonetics). Results for BcPCs relate to TACSI (Terumo BCT) system and a manual pooling and extraction process. For FFP, results relate to either whole blood derived or apheresis plasma, secured by three alternative methods; quarantine, Methylene Blue or Intercept treatments. Our study emphasizes how each production process affects the final quality of the product. For example : total hemoglobin content (g/unit) is higher whether the unit is issued from whole blood filtration (60 ± 7), BAT and RCC filtration (53 ± 6) or erythrapheresis (48 ± 3). Percentage of non-conforming units for hemolysis (>0.8%) at the end of storage is higher for whole blood filtration units (8.6%) than BAT RCC filtered units (3.0%). SDP Platelet content (10 e11/U) is higher when collected on Amicus (5.3) than Trima (5.0) and MCS+ (4.2). Intercept treatment contributes to lower the platelet content of 0.6.10e11 platelets/unit. FVIII content (UI/mL) in FFP is higher in units issued from apheresis (1.10 ± 0.35) than from whole blood (0.90 ± 0.27) but is also altered by Intercept pathogen reduction treatment (0.80 ± 0.30).
These characteristics and their relative importance in terms of quality, safety and clinical efficiency will be discussed.
Summary/Conclusions: Final blood product characteristics are greatly affected by technological choices and process strategies. Blood product quality control monitoring and expertise is a critical tool to assess new technologies as well as process improvements and to assist strategic decisions regarding future technologies and processes.

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QUANTIFICATION OF TRANSFUSED PLATELETS IN RECIPIENTS WITH DROPLET DIGITAL™ PCR COMPARED TO QUANTITATIVE REAL-TIME PCR

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Background: Determination of recovery and survival of transfused platelets without a need for labelling procedures is feasible by detecting genetic markers located in the mitochondrial genome. Quantitative real-time PCR (qPCR) is established as reliable tool for the quantification.
Aims: Droplet digital PCR (ddPCR) was evaluated as an alternative to qPCR for absolute quantification of transfused platelets.
Methods: Platelet rich plasma was prepared from 5 ml blood sample for automated extraction (MagnaPure Compact®) of mitochondrial DNA (mtDNA). Dilution series were analyzed to assess the sensitivity of DNA extraction. ddPCR for mitochondrial markers (n = 8 SNP alleles) was tested for linearity, sensitivity and reproducibility. Blood samples from 30 patients with haemato-oncological diseases were collected for up to six days after platelet transfusion. Endogenous and exogenous platelet counts measured by ddPCR were compared to qPCR results from identical samples.
Results: MtDNA levels increased linearly with platelet counts in the range of 1–50 plt/nl (r = 0.997). Spiking experiments demonstrated a sensitivity of 0.1 exogenous plt/nl in a background of 20 endogenous plt/nl (20 droplets/assay as cut-off value). The coefficients of variation for ddPCR assays were comparable to qPCR: 0.7–3.2% (intra-assay) and 1.7–9.9% (inter-assay) depending on the SNP-allele. Calculated survival times in samples from 30 patients agreed well. The coefficient of correlation for the comparison of platelet counts measured with ddPCR to qPCR was R^2 = 0.96 (all samples) and R^2 = 0.86 (samples with platelet counts up to 10/nL), respectively.
Summary/Conclusions: Our results demonstrate the reliability of ddPCR for quantitative tracking of transfused platelets against a background of endogenous platelets. The limit of detection for transfused platelets is about ten-fold lower than for qPCR. The ddPCR introduces a highly sensitive and cost-effective alternative to qPCR for monitoring platelet transfusions.

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WHOLE BLOOD DERIVED SINGLE-DONOR PLATELET CONCENTRATES FROM DONORS USING NON-STEROIDAL ANTI-INFLAMMATORY DRUGS

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Background: Buffy coats (BC) from donors who used pain medication like aspirin, diclofenac, ibuprofen and naproxen up to 4 days prior to the donation are discarded, because a known side effect of these non-steroidal anti-inflammatory drugs (NSAIDs) is inhibition of platelet aggregation. These NSAIDs inhibit the enzyme cyclooxygenase-1 in a reversible or irreversible manner, thereby blocking synthesis of thromboxane A_2 from arachidonic acid. However, the quality of platelet concentrates (PC), prepared from this BC is not known.
Aims: To investigate the *in vitro* quality, in particular the aggregation and coagulation properties, of PC prepared from NSAID-BC and autologous plasma during storage.
Methods: Single-donor PC (sPC, n = 18) were prepared from 1 NSAID-BC and 60 ml of plasma. Information about the type of pain medication was extracted from the anamneses form. The sPC were stored for 8 days on a flatbed shaker at 22 ± 2°C in a 600 ml PVC-DEHP container and sampled at regular intervals for determination of the *in vitro* quality. Aggregation tests were performed with Chronolog (ADP or collagen) and Multiplate (arachidonic acid) aggregometers. Thromboelastography (TEG), using kaolin as an activator, was applied for assessment of the overall clotting capacity. In addition, sPC (n = 5) in plasma from normal controls were investigated as a reference. Values are expressed as mean±SD or as median, IQR. A non-paired t-test or a Mann-Whitney U test was applied for statistical analyses of normal or non-normal distributed data respectively.
Results: Volume (69 ± 4 vs. 66 ± 16 ml) and platelet (PLT) content (67 ± 14 vs. 71 ± 15 × 10^9) were similar in both groups. At the end of storage, both groups showed comparable pH and changes in platelet content (data not shown). Phosphatidylserine exposure (Annexin V binding) on Day 8 was significant higher in a subset of donors who had used ibuprofen (n = 5). Aggregation tests with arachidonic

acid revealed in general a low or absent response for sPC with aspirin (0.0–30, $P < 0.05$), diclofenac (31.1–76) and naproxen (0.0–24, $P < 0.05$), compared to normal controls (76.64–85). No differences were detected in aggregation with ADP or collagen. In TEG, slightly longer R-times (initiation phase) were measured on Day 1 in sPC with aspirin, diclofenac and naproxen, compared to the normal controls (only significant for naproxen). These differences disappeared during storage.

Summary/Conclusions: Storage properties of sPC prepared from NSAID-BC were comparable with sPC from normal controls. Main differences were observed in aggregation and coagulation properties for donors who used aspirin, diclofenac or naproxen. PLT from donors who used ibuprofen showed little or no deviations. This is most likely caused by the fast (<24 h) disappearance of ibuprofen from the blood circulation and the reversible binding to PLT. The use of BC from donors who used ibuprofen will be further investigated in a 'worst case' (PC in plasma) and 'best case' (PC in additive solution) scenario. The effects of ibuprofen on aggregation and coagulation properties will be further investigated in a dose-response study design adding different levels of ibuprofen to PLT.

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EVALUATION OF DONOR CHARACTERISTICS OF LIPEMIC WHOLE BLOOD DONATIONS

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Background: Several studies on the impact of lipemic plasma on the quality of blood products indicate that lipemic plasma has an adverse effect on the quality of platelets and red blood cells during storage. As a follow-up on those studies, a possible relationship between donor characteristic and the occurrence of lipemic plasma was examined.

Aims: The aim of this study was to expand the knowledge of donor characteristics in relation to the quality of blood products.

Methods: From whole blood donations with lipemic plasma, erythrocyte concentrates in SAGM were produced and *in vitro* quality during cold storage was determined. In addition, donor characteristics were obtained. These data were used to investigate whether there is a relationship between certain donor characteristics, the triglyceride level in lipemic plasma and the hemolysis during storage. Quality control data of normal erythrocytes were used as control group (n = 74).

Results: 26 donations with lipemic plasma were evaluated. The data indicated no direct relationship between the plasma triglyceride concentration and the degree of haemolysis during storage. In 21 out of 26 lipemic donations, haemolysis at day 42 of storage was > 0.4% while in the control group all the units had haemolysis below 0.4%.

Only two lipemic donations were obtained from a female donor, while in the donor population more women are registered as active donor.

The average age of donors in this study was 50 years, with a range from 21 to 67 years.

The average BMI was 26; 8 donors were in the normal range (BMI 18.5–25); 14 donors showed overweight (BMI 25–30), and 4 donors were obese (BMI> 30).

The donations were drawn between 2:40 and 8:40 PM. These included four donations collected before 6:00 PM, seven donations between 6:00 PM and 7:30 PM and 11 donations after 7:30 PM. It should be noted that at our blood bank most blood donations take place in the evening.

The normal triglyceride concentration in blood is ≤1.7 mmol/l. The triglyceride concentration in the lipemic plasma units was on average 5.9 mmol/l. The lowest triglyceride level was 3.3 mmol/l, while 3 units showed a triglyceride concentration >10 mmol/l. These 3 units were donated after 8:00 PM.

Summary/Conclusions: In 81% of erythrocyte concentrates made from whole blood with lipemic plasma, haemolysis was greatly enhanced during storage. Based on the limited set of donations in this evaluation, a tentative conclusion is that highest chance of lipemic plasma exists in donations from men over 50 years with BMI >25, donating in the evening. More donor characteristics from donors related to lipemic units, which are rejected for production of blood products, should be collected to perform a more extensive data analysis.

Based on these investigations, our blood service decided not to use lipemic whole blood for the production of (cellular) blood products. Donors will be provided with instructions how to prevent lipemic donations as much as possible.

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NUMERICAL INVESTIGATION OF RED BLOOD CELL MEMBRANE MECHANICAL PROPERTIES AND DEFORMABILITY DURING INDENTATION

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Background: The deformability of red blood cells (RBCs) is vital to their function as they must deform significantly and repeatedly to pass through narrow capillaries. Deformability is largely governed by the mechanical properties of the RBC membrane, composed of an outer plasma membrane and cytoskeleton tethered beneath. Structural transformations and abnormalities within the membrane can alter the deformability of the RBC, which is known to occur during storage. RBC deformability can be quantified experimentally by measuring the force-deformation response during indentation with atomic force microscopy. This technique has been shown sensitive enough to detect a difference in membrane stiffness between healthy and deteriorating cells. However, these experiments have not been able to provide deeper insight into the underlying mechanisms which cause the measured differences. Numerical modelling can be used as a complementary tool for exploring how the mechanical characteristics of the RBC impact deformability. Findings from these numerical studies can then be linked back to more complex biological hypotheses, which may in turn guide further studies for preventing the loss of deformability to RBCs in storage.

Aims: This study aimed to investigate the influence of RBC membrane mechanical properties on RBC deformability as measured during indentation, using a coarse-grained particle method (CGPM) model.

Methods: The RBC was modelled using the CGPM which involved discretising the membrane into a series of particles interconnected by a network capable of storing energy. Relationships between particles were developed to model the mechanical behaviour of the cell – membrane bending resistance, membrane incompressibility, and volumetric incompressibility of the internal fluid. A stiffness coefficient was associated with each energy-storing mechanism to control its strength. The simulation was evolved by moving particles to minimise energy stored in the membrane, leading to a prediction for the cell's preferred shape. Cell shape and force-deformation response were validated against experimental data obtained using a 5 μm spherical probe. A parametric study was then conducted on the stiffness coefficients used in the CGPM model to investigate which mechanisms had the most significant impact on deformability.

Results: Varying the membrane's bending stiffness caused a substantial change in the deformability of the RBC. When the bending stiffness was increased tenfold, the force needed to indent it to a nominal depth of 200 nm was 5 times greater. When the bending stiffness decreased tenfold, just over a tenth of the original force was required. Modifying the incompressibility of the membrane impacted deformability to a lesser extent, with a tenfold increase requiring just 1.4 times additional force and a tenfold decrease requiring approximately half. Furthermore, the effect of increasing membrane incompressibility began to plateau, meaning further increases had little effect on the RBC's deformability.

Summary/Conclusions: The deformability of the RBC was most sensitive to the membrane's bending resistance, which is provided by the plasma membrane. This suggests that changes within its structure, such as clustering of band 3 proteins due to oxidative damage or the change in lipid distribution over time, play the most critical role in deformability loss and should be the focus of further investigation.

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APHERESIS PLATELET CONCENTRATES VS BUFFY-COAT-DERIVED POOLED PLATELET CONCENTRATES: FOCUS ON SCD40L AND SCD62P

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Background: Platelet (PLT) storage lesions describe the structural and biochemical changes in PLTs and depend on production methods, on platelet additive solutions (PAS), on pathogen inactivation method, and on storage container and storage duration.

Aims: This study investigated sCD40L and sCD62P release by Apheresis platelet concentrates (SDA-PCs) and Buffy-coat-derived pooled platelet concentrates (PPCs).
Methods: Near 9,000 samples were investigated, depending on preparation and storage duration. SDA-PCs and PPCs were produced in one regional setting of the National Blood Service EFS. Soluble factors were quantified in the PC supernatants using Luminex technology.
Results: SDA-PCs appear more activated than PPCs at the end of the preparation stage, i.e. prior to storage. However, proinflammatory soluble factors, in PPCs are more elevated along storage than for SDA-PCs. In SDA-PCs, the concentration of sCD62P is smoother with Intersol™ than with SSP+®, and vice-versa for sCD40L.
Summary/Conclusions: Data stress out the importance of processing and storage. To what extent do they affect patient outcome remains to be evaluated in clinical studies.

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PLATELET FUNCTION DURING STORAGE – ASSESSED BY A NOVEL FLOW-CYTOMETRIC PLATELET AGGREGATION ASSAY

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Background: Platelet concentrates are widely used to treat and prevent bleeding in thrombocytopenic patients. A novel flow cytometric platelet aggregation capacity assay allows for measurement of platelet function independent of platelet count and the platelet suspension medium. Thus, the assay may be used to measure platelet function in blood products.
Aims: To investigate the impact of the preparation method and storage conditions on platelet function in different blood products as measured by flow cytometric platelet aggregation capacity.
Methods: Four buffy-coat derived platelet concentrates (BPC), 1 unit of apheresis platelets (AP) and 2 whole blood (WB) units were obtained from qualified blood donors. The BPC and AP were stored for 9 days, at 20–24°C on a flatbed agitator, while WB units were stored at 2–6°C. Samples were taken from WB and BPC on day 1, 2, 5, 7 and 9 (WB also on day 15) and from AP on day 0, 1, 4, 6 and 8.
For the flow-cytometric platelet aggregation assay, platelets were isolated by centrifugation. Then, two fractions, with cell counts of 144×10^9/L and 16×10^9/L, respectively were produced and labelled with calcein AM and calcein Violet 450 AM, respectively. The two fractions were incubated with plasma for 30 min in order to mimic in vivo conditions following transfusion. Activation was initiated by addition of thrombin receptor-activating peptide (TRAP) and shaking the sample for 5 min at 1,000 rpm. Platelet function was assessed in triplicate by flow cytometry (BD FACSCanto II) as the percentage of double positive calcein AM and calcein violet 450 AM events out of all the calcein violet 450 AM positive events. The normal range of platelet aggregation capacity by this assay is 63–67% in healthy blood donors.
Results: On day 0, AP exhibited significant higher platelet aggregation capacity compared to both BPC and to WB on day 1 (mean aggregation: 59% vs. 46%, P = 0.045 and mean: 59% vs. 41%, P = 0.01 BPC and WB, respectively). There were no significant differences between WB and BCP on day 1 (P = 0.5).
For all 3 products, platelet aggregation capacity declined during storage. A significant reduction compared to day 1 was detected on day 5 for BCP (mean: 46% vs. 34%, P = 0.0007), on day 15 for whole blood platelets (mean: 41% vs. 31%, P = 0.0001), and for AP aggregation capacity declined after 1 day in storage (mean: 59% vs. 47%, P = 0.001).
On day 7 BCP exhibited significantly lower platelet aggregation capacity compared to WB day 7 and to AP day 6 (mean: 28% vs. 41%, P = 0.0003 mean: 28% vs. 37%, P = 0.04 WB and AP, respectively). The difference between WB day 7 and AP day 6 was not significant (P = 0.7).
Summary/Conclusions: Measured flow cytometrically by TRAP-stimulated platelet aggregation capacity, AP initially exhibits the highest platelet function, but declines faster than WB and BCP. WB platelets and BCP have comparable levels of platelet function at the time of production. However, WB platelets appear to be more stable over time than either AP or BCP.

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ADDING TO PLATELET SAFETY AND LIFE: PLATELET ADDITIVE SOLUTIONS

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Background: Platelet Additive Solutions (PAS) are crystalloid nutrient media used in place of plasma for platelet storage. They replace 60–70% of plasma in platelet components, so the amount of storage plasma can be decreased.
Platelets in PAS have lower risk for allergic transfusion reactions with equivalent clinical efficacy for controlling bleeding, compared to platelets stored in plasma. Transfusion of PAS platelets vs plasma could result in a lower incidence of plasma-associated transfusion reactions like allergic reactions, ABO hemolytic reactions and transfusion-related acute lung injury (TRALI).
Aims: To analyze the clinical & laboratory efficacy of PAS-Platelets.
Methods: Rotary Bangalore TTK Blood Bank, collects 35,000 blood units annually from voluntary donors only and performs nearly 2,500 Single Donor Platelets (SDP) donations yearly. As a Policy blood bank intended to collect all SDPs also from voluntary blood donors & get the NAT testing done. Due to short shelf life & high cost, maintaining inventory of SDPs units was a challenge.
The Blood Bank started using Apheresis Platelets with PAS (SSP+, MacoPharma, Mouvaux, France) for better inventory management and to facilitates ABO incompatible SDP transfusion. The objective of this study is to perform validation of SDP-PAS and also to analyze its clinical outcome. Total 450 SDP collected in PAS in June & July 2016 by two different Apheresis systems: Amicus (n = 400) and Trima Accel (n = 50). Amicus has automated programming for PAS but in Trima, SDP was collected as concentrate & then PAS was added with sterile connecting device. The PAS to Plasma ratio was 70:30.
The parameters analyzed were antibody titer of Anti-A & Anti-B, Volume, Platelet count, pH, Bacterial contamination and reporting of adverse transfusion reaction.
Results: For Antibody Anti-A Titer in SDP with PAS units: 26 samples were negative, 64 samples were 1:1, 86 samples were 1:2, 134 samples 1:4, 86 samples 1:8, 54 samples 1:16 and 18 samples 1:32. For Antibody Anti-B Titer in SDP with PAS units, 22 samples were negative, 52 samples were 1:1, 96 samples 1:2, 126 samples 1:4, 72 samples 1:8, 48 samples 1:16 and 20 samples 1:32. Out of total 450 SDP units 21 SDP's Platelet Count was $< 3.0 \times 10^{11}$ platelets/unit, 218 SDP's Count was between $3.0–4.0 \times 10^{11}$ platelets/unit and 211 SDP's count was $>4.0 \times 10^{11}$ platelets/unit. The mean platelet count was 3.6×10^{11} platelets/unit with range of $2.8–5.8 \times 10^{11}$ platelets/unit. The mean volume of the SDP collected was 263 ml & the range was 200–300 ml. Swirling was present in all the SDP Units and pH of all the SSP units was >6. All the units found negative for bacterial contamination. No Transfusion Reaction was reported of the units transfused.
Summary/Conclusions: Transfusion of only ABO-compatible platelets is not always feasible due to limited availability and maintaining the daily SDP inventory is a challenge for most of the blood banks.
The ABO antibody titers were significantly reduced after addition of PAS. This facilitates the ABO incompatible SDP transfusion and help in inventory management.
The risk of allergic transfusion reaction decreases after reducing the amount of plasma from SDP units.

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BLOOD BANKING OF RARE BLOOD IN A UNIVERSITY HOSPITAL IN GREECE

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Background: A challenge in transfusion medicine is to provide compatible blood for patients who are negative for blood group antigens with a very high frequency in the population (common antigens), as well as positive for alloantibodies against any of those antigens. A second category of patients for whom compatible blood may be difficult to find are those with a combination of alloantibodies, so that donors negative for all the antigens involved are very rare. So rare blood is characterized as one which lacks a high frequency antigen or lacks multiple common antigens.
Aims: To show the usefulness of testing blood donors for rare blood groups and keeping the records for future use in some patients mainly multitransfused with thalassemia and sickle cell anemia.
Methods: From the year 2003, donors of O, A and B group, D+ with phenotypes Ccee, CCee, ccEe, ccee and also D- with phenotype ccee are tested for the antigens Kell, Cw, Jka/b, Fya/b, M, S, s by solid phase test (Galileo, Immucor) and for Kpa/b,

Lea/b, P1, Lua/b by gel test (Diamed). Kell positive donors are tested for k(cellano). The results are recorded electronically for future use. When a rare blood type is requested for a patient with a single or multiple red cells antibodies, the database can be searched to locate donors negative for a high-prevalence antigen or negative for multiple common antigens to ensure the patient receives compatible blood. Every day the new donors are tested for the above mentioned antigens. For our patients with repeated regular transfusions and known red cells antibodies we store units with corresponding antigenic profile and in cases of shortages we call the appropriate donor.

Results: The period 2003–2016, 13,150 donors were tested for these antigens. 105 patients with single or multiple antibodies (except anti-D,E,e,C,c) have been transfused with 3,590 selected RBCs units. 23/105 patients suffering from thalassemia and sickle cell anemia and have regular transfusions. 768 units of rare blood were sent to other hospitals in the country in order to transfuse their patients. The rarer combinations of multiple absent red cell antigens are as follows: Kell-Cw-Kpa-Fya-Jka-Leb-Lua/ Fyb-s-/ Fya-Jka-s-/ Kell-Cw-Kpa-Jka-S-Lea-Lua /Fyb- Jkb-/ Kell-Cw-Kpa-Fya-Jkb-S-Lua-/Fya-S-.We also found 44 donors who lack a high frequency antigen: 8 Lub-,2 Kpb-,2 Kpa-b-, 27 k(cellano)-,5 Fya-Fyb-.

Summary/Conclusions: Donors with rare blood group are especially important to patients who have complex blood compatibility problems. As a result of our efforts in donor screening, our Blood Bank has been successful in providing for the needs of our patient population (mainly those with thalassemia and sickle cell anemia) as well as the needs of many problematic patients across the country.

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A COMPARATIVE STUDY OF QUALITY CONTROL PARAMETERS OF RANDOM DONOR PLATELETS STORED IN DINCH-PVC CONTAINER VS CONVENTIONAL DEHP-PVC CONTAINER AFTER 5 DAYS

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Background: Having the property of reduction of red cell hemolysis and low manufacture cost, DEHP-PVC blood containers have been commonly used in whole blood collection and blood components storage over the last five decades. The disadvantages of relatively poor gas permeability and leaching of DEHP into the component contents not only affect the quality of platelet, but also impose health concerns such as endocrine disruption particularly in young male patients, such that reducing exposure to DEHP and replacing DEHP with alternative plastics are recent interests in blood transfusion. PVC plasticized with diisononyl cyclohexane-1,2-dicarboxylate (DINCH) is one of the newer material for manufacturing of platelet containers.

Aims: In this study, random donor platelet units prepared in commercial platelet containers made of DINCH-PVC were evaluated and compared with those prepared in conventional DEHP-PVC containers.

Methods: Quality control results of random donor platelet concentrate units prepared from 450 ml whole blood donations during the period from May 2016 to October 2016 were retrieved from our Blood Bank Quality Control Database. A total of 95 platelet concentrate units were prepared from whole blood collected in 450 ml quadruple blood bag system (Composelect, Fresenius-Kabi, Bad Homburg, Germany) and suspended in plasma in DINCH-PVC containers, whereas 626 platelet concentrate units were stored in conventional DEHP-PVC containers (JMS, Singapore). Platelet concentrates prepared from platelet rich plasma were separated by semi-automated blood extractor (CompoMat G5, Fresenius-Kabi, Bad Homburg, Germany). At day 5, end of platelet shelf life, quality parameters of the platelet units were measured, which included pH by PB-20 pH meter (Sartorius, Goettingen, Germany) and absolute platelet count by Unicel DxH800 Analyser (Beckman Coulter, Miami, FL, USA). Results were statistically analysed by the z-test and P-value was set at 95% significance level.

Results: pH measured at 22°C with a value >6.4 (Council of Europe standard) was used as the quality indicator. 98.9% of platelet units in DINCH-PVC containers achieved pH>6.4, whereas 89.3% of those in DEHP-PVC containers attained the standard ($P = 0.0027$). The mean pH at Day 5 was significantly higher in DINCH-PVC containers (mean $\pm$ 2SD = 7.30 $\pm$ 0.44) than that in DEHP-PVC (mean $\pm$ 2SD = 6.98 $\pm$ 0.94) ($P < 0.0001$). There were no differences in platelet counts between platelets preserved in DINCH-PVC and DEHP-PVC bags at day 5.

Summary/Conclusions: The present study showed that a higher percentage of platelet concentrates demonstrated better pH when stored in DINCH-PVC than in DEHP-PVC containers after a preservation period of 5 days, which can be attributed to the improved gaseous permeability property of the former plastic. DINCH-PVC is a suitable alternative to DEHP-PVC for platelet storage and would reduce DEHP exposure to patients during blood transfusion.

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EVALUATION OF RBC QUALITY FROM WHOLE BLOOD PROCESSED AT TWO DIFFERENT SPEEDS ON THE NOVEL BLOOD CENTRIFUGE MACOSPIN

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Background: More than 80,000 units of whole blood (WB) and 5,000 units of buffy-coat platelet-pools are centrifuged annually at the Unit of Blood Component Production at Karolinska University Hospital (Stockholm, Sweden). When a demand for new centrifuges was identified, the novel centrifuge MacoSpin (MacoPharma, Mouvaux, France) was considered.

Aims: The aim was to compare centrifugation of WB on MacoSpin to our centrifuge Sorvall RC12BP (Thermo Scientific, Waltham, MA, USA). We wanted to evaluate both a centrifugation program mimicking the Sorvall program, plus a harder, optimized, centrifugation program. In this study we concentrate on quality of RBCs.

Methods: 12 blood collection packs of BAT system NPT6280LE (MacoPharma) containing 450 ml $\pm$ 10% WB + 63 ml CPD were centrifuged in *(i)* Sorvall, 2,988 $\times$ *g*, 10 min (Sorvall), *(ii)* MacoSpin, 3,130 $\times$ *g*, 11 min (MacoSpin regular, mimicking Sorvall centrifugation) and *(iii)* MacoSpin, 4,900 $\times$ *g*, 11 min (MacoSpin hard).

WB was processed in MacoPress Smart Revo (MacoPharma). 100 ml SAG-M was added to the RBCs to create a red cell concentrate (RCC) which was leukoreduced and placed in 4°C-storage within 8 h of donation. From each centrifugation, 10 RCCs were randomly chosen for the study. Hemoglobin, hematocrit, glucose, lactate, pH, extracellular potassium, haemolysis and ATP were measured on day 1, 8, 15, 22, 29, 36 and 43. 2,3-DPG was measured until day 21. Results were compared between the different centrifugation parameters.

Results: Centrifugation parameters did not affect hematocrit or hemoglobin, which were stable during the duration of the storage time.

Levels of glucose, ATP and extracellular potassium, as well as pH, were unaffected by centrifugation parameters. Glucose and pH decreased over time while potassium increased. ATP increased slightly on day 8 and decreased thereafter.

Lactate started at similar levels and increased over time. On day 29, Sorvall started displaying a slight but significant increase compared to MacoSpin hard. The difference persisted throughout the storage period.

Significantly higher haemolysis was observed for MacoSpin Hard compared to the other centrifugations in the beginning of the storage time. However, from day 28 and onward, no significant differences were displayed. Haemolysis increased over time, ending at 0.34 $\pm$ 0.07% for Sorvall, 0.36 $\pm$ 0.10% for MacoSpin regular and 0.36 $\pm$ 0.07% for MacoSpin hard.

Levels of 2,3-DPG decreased rapidly over time and were undetectable on day 21. Significantly lower levels of 2,3-DPG were observed for MacoSpin hard compared to MacoSpin regular on days 8 and day 15.

Summary/Conclusions: The most protruding difference was the slightly higher haemolysis of MacoSpin hard directly after centrifugation. Differences had evened out by day 28 and were very low (below half of the maximum allowed) for all three series at the end of storage.

Lactate levels were slightly elevated for Sorvall at the end of storage. 2,3-DPG rapidly decreased for all centrifugations, but more rapidly for MacoSpin hard. However, since neither ATP nor pH were affected, the reduction of RCC quality is probably negligible.

We conclude that MacoSpin is a satisfactory alternative as a blood bag centrifuge, both at close to maximum speed and below.

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CORRELATION BETWEEN ABSENCE OF SWIRLING IN PLATELETS CONCENTRATES AND BIOCHEMICAL CHANGES IN METABOLISM

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Background: Apheresis platelets (AP) and whole blood platelets concentrates (WBP) are suspended in 65% of PAS-E and treated by Intercept Blood System for pathogen inactivation (PI). Platelets concentrates (PC) stored at 22 ± 2°C for 5 days are monitored daily for presence of swirling. Some PC (exclusively AP) are discarded for absence of swirling (AOS) as marker for loss of functional properties. We systematically investigate AOS AP for different biochemical parameters in order to identify critical steps leading loss of functional properties evidenced by AOS.
Aims: Confirm correlation between AOS in PC with changes in metabolic profile and identify potential root causes.
Methods: Swirling parameter was controlled daily every morning on 100% of the PC inventory. Samples were drawn on detected AOS PC for pH, pCO2, pO2, glucose and lactate measurement on a GEM 3500 analyser. Platelets concentrations [PLT] were measured after PI. Collection and process data of AOS AP were reviewed and correlated with biochemical parameters.
Results: On a total of 10,538 WBP produced and 11,223 AP collected in 11 collection sites in 2016, no WBP and 92 AP were discarded for AOS representing 0.9% of the AP collected from 78 different donors. This defect was detected in all 11 sites with frequencies ranging from 0.1% to 1.3% of the collected AP. AOS was detected between day 2 to day 5 (median: 3). 4 donors out of the 78 had ≥3 donations discarded for AOS. No significant process deviation could be incriminated as root cause for AOS. pH in AOS AP was 6.59 ± 0.13 vs. 6.98 ± 0.06 in control group (C, N = 6, day 3), [glucose] was at 0.7 ± 1.1 mmol/l vs. 3.95 ± 0.90 in C, [lactate] was at 16.3 ± 3.3 mmol/l vs. 6.6 ± 1.2 in C, pCO2 was 40 ± 10 mmHg vs. 26 ± 4, pO2 was 58 ± 24 mmHg vs. 105 ± 12 respectively in C. For [PLT] between 800 and 999 E + 3/µl AOS frequency was stable at 2.2%; for [PLT] between 1,000 and 1,499 E + 3/µl AOS frequency was 0.4% and raised progressively up to 4.8% between 1,500 and 2,000 E + 3/µl reaching 8.5% for atypical collection procedures with AP with [PLT] >2,000 E + 3/µl. For [PLT] ≥ 1,300 E + 3/µl, we observed a correlation between [PLT] in AP and frequency of AOS (R^2=0.95).
Summary/Conclusions: AOS detected metabolic changes during storage and allowed discard of non-functional PC. Analytical results confirmed changes in metabolism during storage of AOS AP probably due to activation of the platelets. We observed a correlation between sub-optimal [PLT] (outside 1,000–1,499 E + 3/µl) and higher frequency of AOS. We detected a limited number of donors giving platelets with high metabolic rates resulting in AOS before day 5 of storage. One donor regularly used food supplements that could influence PLT metabolism. We are further investigating donors for dietary habits. No other process steps could be incriminated in AOS AP and additional investigations are required to confirm other potential root causes.

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Abstract has been withdrawn.

P-221

INFLUENCE OF PROPER NUTRITION TO THE QUALITY OF BLOOD COMPONENT

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Background: Blood components are blood products prepared for directed blood transfusion. The component therapy has significant logistics, ethical and therapeutic advantages. The quality of blood component is highly influenced by proper nutrition, since the blood of a person with proper diet represents, among other things, a safe medication for the patient. Proper nutrition is the best health method and manner of preventing numerous diseases.
Aims: Aim of the paper is to show in what way proper/improper nutrition affects the number of lipemic blood components through a comparative review of January – June 2015/2016 period, after providing education to blood donors through a nutrition form created by the Institute in October 2015, which is the starting date of its application.
Methods: We used a comparative analysis of a computer method in the form of comparative report on the produced blood components. The research covers the 6-month period of 2015/2016. Results of the survey have been presented as numerical data in the form of graphics.
Results: The analysis covers produced components in the period of the first six months of 2015/2016. The following input data is available: total number of components produced in 2015: 18,302 and the number of components in 2016: 17,041. Number of blood donors dropped in 2016 so the number of produced components is lower by 17.8%. Number of lipemic blood components in 2015: 668 and in 2016: 345. Even though the number of produced components was reduced by 17.8%, the introduction of nutrition form has significantly reduced the number of lipemic components and therefore the number of rejected dosages was reduced by 48.4% as well. This percentage would definitely be higher if the attention had been paid, besides providing education to the nutrition of blood donors, to collecting blood samples in the morning instead of afternoon after a large meal.
Summary/Conclusions: Providing education to voluntary blood donors through nutrition form before and after donating blood certainly affects the quality of blood as a cure. Apart from this, it influences the condition of the blood donors after donating their valuable liquid because each pleasant donation and condition after it is a sign showing if the donor would come back to donate blood again. It is stated in the nutrition form that proper nutrition is a significant aspect in the chain of obtaining a good-quality blood component. We hope that our next analysis will give even better results that will show less lipemic components and the possibility of appropriate serologic test.

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SMOOTH FILTRATION FLOW -QUALITY ISSUE – HOW TO OBSERVE IT?

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Background: Approximately 85 million units of red blood cells (RBCs) are transfused annually worldwide. Most of produced RBCs are issued for haematology and oncology patients, usually multitransfused patients. Modern transfusion therapy optimize blood care with advanced technology, processing standardised blood components. Leukocyte contamination during blood transfusion can cause many adverse effects (Febrile non-haemolytic transfusion reaction, transmission of cell associated infectious agents, alloimmunization, platelet refractoriness, immunosuppression etc.). RBCs, Leucocyte-Depleted (LD) are red cells component derived from Whole Blood donation, Red Cells or RBCs-BCR (buffy coat removed), by removing the leucocytes. RBCs- LD contains less than 1.0×10^6 leucocytes.
Aims: Our goal are well chosen standardised, LD cellular blood components. In order to process good quality components, our choice were filtered RBCs, either with "in line" soft filters for RBCs – inline pre-storage filtration, or postproduction filtration with dockable filters, post-storage laboratory filtration. All filters have high Red Cell recovery and transparent housing for visual monitoring. In order to assess the impact of filtration flow (duration time, flow chart) and to visualise the process itself, we used computerized device- Filtramatic® (LMB technologies).
Methods: Blood was collected in top & top blood bags with "in line" soft filters for RBCs filtration (LPB System, Leucoflex LCR5 filter, Macopharma) and in top & bottom quadruple blood bags (MQT Systems, Macopharma). Totally 138 blood donation. Whole blood units were processed according to the standardized recommended procedure, into buffy-coat –depleted RBCs in SAGM, leucoreduced either by filtration with "in line" soft filters (LCR5 filters) or with KSV System (LCG2b filters Macopharma) in post production of RBCs BC depleted in SAGM, up to 3 days after collection and in stock on +4°C. This filter with transfer bag has by pass line, to remove air and also to improve recovery.
RBCs, LD were produced: by leucocyte filtration of RBCs after subsequent centrifugation and removal of the plasma and BC. The subsequent leucocyte filtration process was monitored continuously on the device Filtramatic® (LMB technologies) – a device for filtration monitoring and controlling, based on the time tracking against weight reduction. Filtramatic offers different profiles for supporting different filtration processes with multiple adjustable parameters, such as end correction factors for final products. The device also has possibility of integrating all collected information data into the existing quality management information system.
Results: The average donation time with dockable filters was 27:30 min., the average start weight was 361 g and the average filtrated weight was 261 g. The average donation time with "in line" filters was 23:49 min., the average start weight was

293 g and the average filtrated weight was 225 g. Minimum flow during filtration was 2 ml/min and maximum flow was 20 ml/min.
Summary/Conclusions: The Filtramatic® device enables monitoring of the filtration process itself for each RBC component either during process or afterwards. The filtration time and weight measurements are done automatically. The stuff can better observe the procedure. The additional filtration process control, is step more to standardization, complete automatization and haemovigilance.

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IMPACT OF THE REVEOS® SYSTEM FOR WHOLE BLOOD (WB) PROCESSING ON PRODUCTIVITY, BLOOD BANK LOGISTICS AND PROCEDURAL EFFICIENCY

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Background: The ETS "asbl La Transfusion du sang" of Charleroi, Belgium, evaluated the introduction of the Reveos® System (Terumo BCT). It was selected for evaluation because it automates and combines centrifugation and separation in one device. Depending on the protocol, blood components are separated into two (2C) or three (3C) components: plasma, red cells, and an interim platelet unit (IPU) that can be leukoreduced and pooled into one platelet concentrate. The Reveos® System can be used with T-IPU, a software tool that can help increase and/or standardize the pooled platelet concentrate by removal of low-yield IPUs from the pooling process or by offering to combine low- and high-yield IPUs. The yield estimate can also guide the operator in selecting the optimal number of IPUs (usually 4 or 5).
Aims: The results of the introduction of the Reveos® System are presented and discussed focusing on the impact on blood bank logistics and procedural efficiency.
Methods: The former production set-up consisted of semi-automated WB separation with bottom-and-top bags (4 Optipresses + 2 centrifuges) and two apheresis platforms (Trima Accel and Amicus). The new production set-up consists of 3 Reveos® devices with 4 protocols (3C/2C/Overnight/Fresh) + Reveos® System Manager (RSM) and T-IPU, supplemented by one apheresis platform (Trima Accel). In the new set-up a first sort is carried out before separation to determine the protocol (3C/2C). Donations from donors with medical antecedents potentially impacting platelet quality are selected for 2C together with donations collected on Friday and Saturday (no pooling during weekends) as well as group AB or B donations. All other donations from return donors with sufficient platelet counts and acceptable white blood cell levels are used for 3C. This results in 55% 3C and 45% 2C and plasma recovery maximization (ethical and business advantages). It furthermore avoids preparation of pools ultimately noncompliant with release criteria thus maximizing IPU usability (organization and business advantages).
Results: After the deployment of full automation, production evolved from 23% WB derived platelets and 77% apheresis platelets to 65% Reveos® platelets and 35% apheresis platelets. The process has become considerably easier reducing the likelihood of error and shortening training time which indirectly increased flexibility and simplified staff scheduling. Logistical flexibility likewise improved thanks to the 4 production protocols (overnight/fresh/3C/2C) and allowed for improved cell separation with less orange-red plasmas resulting in a more than 90% reduction of plasma bags rejected for color noncompliance (orange-red). Traceability was optimized through RSM. Pooling guided by means of T-IPU resulted in platelet yield optimization. The 2C protocols (overnight and fresh) yielded more plasma volume. The enhancements in logistical efficiency and product output also resulted in net financial advantages.
Summary/Conclusions: As reported in our previous abstract (Pineau, Vox Sang, 2016), the quality parameters of the products ensuing from the migration to fully automated WB separation complied with European guidelines. This secondary analysis demonstrates that the introduction of the Reveos® System has furthermore led to increased flexibility, procedural efficiency and output.

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EVIDENCE BASED PRACTICE OF FRESH FROZEN PLASMA REPEAT FREEZE THAW

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Background: Fresh frozen plasma (FFP) is considered adequate for immediate transfusion after thawing and up to 120 h if kept at 1–6°C. FFP is used to replace deficient clotting factors in patients of trauma, DIC, and Coumadin overdose in various conditions. In small medical centers this policy can lead to excess waste of unused thawed FFP units. Medical literature indicates that Factor VIII is the most sensitive of the clotting factors to freeze-thawing in FFP, were as Free Protein S activity is relatively stable. If clotting factor activity in FFP after a second freeze is within normal range, then the product may meet transfusion requirements
Aims: This study examines Factor VIII and Free Protein S activity levels in twice frozen and thawed FFP units kept at 1–6°C up to 96 h, in order to reduce waste of this limited resource
Methods: Four FFP units of each blood group (A, B, AB, and O) were thawed at 37°C and sampled for Factor VIII and Free Protein S activity levels. The FFP Units were kept for 2 h at 4°C, and refrozen at −80°C. The units were re-thawed after 24 h, sampled, stored at 4°C and resampled every 24 h up to 96 h
Results: The levels of F VIII, and Protein S activity of all FFP units were within the normal range at all six time points measured
Summary/Conclusions: FFP which has been twice frozen and thawed can provide adequate clotting factors, potentially reducing waste of thawed FFP units.

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WASHED PLATELET CONCENTRATES IN BRNO FACULTY HOSPITAL

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Background: Brno Faculty Hospital is the only hospital in Brno and South Moravia region of Czech Republic, which provides patients with basic, specialised and higly specialised care in all medical branches, regardless of the nature of the disease, severity of the accident or patient's age. Washed components are in Czech republic commonly issued to IgA deficient patients or to patients with severe allergic reactions to conventional components. Washed platelets are derived from secondary processing of platelets components with sequential washing and following resuspension of the platelets in the same solution as used for the washing and there is wide variation in this practise. Concentration of proteins should decrease under 0.08 g/l and 10–30% of platelets can be lost.
Aims: Recently we prepared and repeatedly administered washed platelet for two IgA deficient patients in the phase of allogeneic stem cell transplantation. For washing two kinds of platelets were evaluated: apheresis leucocyte-depleted platelets and pooled, leucocyte-depleted platelets, both in additive solution.
Methods: Washed platelets were carried out manually by centrifugation. SSP+ solution was added to bags with apheresis or pooled platelets, both leucocyte-depleted and in additive solution (SSP+). After spinning all the supernatant was removed and only SSP+ solutions in the indigenous volume was added to sediment of platelets. Aggregation and demonstration of swirling phenomenon was carried out 1 h after washing was finished. Prepared platelets then were tested for protein content, pH level and loss of platelets. Shelf life after washing was 24 h.
Results: We prepared 29 units of washed platelets from November 2016 to March 2017. Mean age at time of washing was 3 days, mean content of platelet was 213.10^9. Tested washed products, apheresis or pooled platelets, met the prescribed parameters of protein content, pH level and loss of platelets. For apheresis platelets there was more frequent visible precipitation 1 h post washing, in some cases irreversible.
Summary/Conclusions: Platelets washed in SSP+ solution resuspended without any content of plasma are not suitable for longer storage. It is routinely used not more than 24 h after preparation in Brno Faculty Hospital. Despite to possibility prepared both, apheresis or pooled platelets, we prefer for washing pooled, leucocyte-depleted platelets, in additive solution, because of low risk of irreversible precipitation and without risk of undesirable prolongation of preparing.

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FREQUENCY OF ABO AND RHESUS BLOOD GROUP IN A COSMOPOLITAN CITY – PAKISTAN

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Background: Karachi Pakistan is a cosmopolitan city with multiple ethnicities and races. Previous studies of blood group distribution in Pakistan have been focused to specific areas with predominance of single ethnicity.

Aims: To investigate the frequency of ABO and Rhesus blood groups in healthy blood donors of multiple ethnicities in Pakistan.
Methods: ABO and Rh blood grouping was performed on 45,558 blood donors by tube method. Weak D testing was performed on all Rhesus negative blood donors.
Results: Out of 45,558 blood donors analysed, 93% were Rhesus Positive and 7% Rhesus Negative. The ABO group distribution for Rhesus positive was B (32.9%), O (29.5%), A (22.0%) and AB (8.6%). Furthermore, for Rhesus negative, the ABO distribution was B (2.5%), O (2.2%), A (1.6%) and AB (0.7%).
Summary/Conclusions: The frequency of ABO and Rhesus blood groups among the donor population of Karachi was B > O > A > AB. The frequency of Rhesus negative in the Pakistani population is low.

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ANALYSIS OF DISCARDED AND WASTAGE BLOOD IN A BLOOD TRANSFUSION CENTER OF SPAIN IN A FOUR YEARS PERIOD (2013–2016)

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Background: Optimizing blood collection and processing would reduce the rate of discard and improve the efficiency of the Blood Transfusion Center (BTC), this point have financial and ethical challenge in the daily BTC. So we must to analyze our dates about the different causes of discard and wastage of blood components and then to take steps to improve processes to achieve the maximum quantity and quality of safe blood with the better efficiency
Aims: To determine the rate and number of discarded whole blood & apheresis components, causes of wastage and number of blood components processed. Data were obtained from the BTC Toledo-Guadalajara Information System (IS), from January 2013 to December 2016.
Methods: During the period studied, we analyzed the blood components rejected, reasons for discard including outdated blood, and area associated (Selection; Extraction; Laboratory; Processing; Storage) using the IS Delphyn 8.0.9. All data were registered in a database Excel 2010 (Microsoft Corporation USA). The statistic analysis were performed with MedCalc 12.2.1.0 P value <0.01 was considered as statistically significant.
Results: In the four years period we have 107,470 whole blood (WB) donations, 2,126 Multicomponent apheresis donations (MC-platelets & MC-plasma) and 4,670 Plasmapheresis (PLP) donations. Insufficient WB at the donation moment was 687 (0.64%), there was not statistical differences between years.
During the period studied the components produced were 104,864 Red Blood Concentrates (RBC), 104,812 WB-plasma, 71,531 Buffy Coats (BC), 12,237 Pool Platelets of BC (PP), 2,198 MC-Platelets and 6,764 Apheresis-Plasma (Ap-PLP). Donations and components produced were similar in the 4 years period.
Blood components discarded were 831 (0.77%) WB, 1,509(1.44%) RBC, 2,425(2.31%) WB-Plasma, 8,267 BC, 220(1.8%) PP, 52(2.36%) MC-Platelets and 379(5.6%) Ap-PL. There were not statistical differences of global rate of discard between years. The rate of the different components discarded at the four years period was similar.
The main area associated at the wastage by blood component was: **WB**- 718(0.66%) at DA and 86(0.08) at the PA; **RBC**- 1,087(1.04%) outdated and 229(0.22%) at the PA; **WB-Plasma**- 1,664(1.59%) at the LA and 596(0.57%) at the PA; **BC**- 7,369(10%) at the PA and 1,171(1.64%) at the LA; **PP** 166(1.35%) at the PA; **MC-P**- 32(1.45%) at the DA; **Ap-PLP**- 272(4%) outdated and 87(1.3%) at the PA.
The percentage of areas affected for the global rate of blood components discarded were 9.96% Outdated, 5.18% DA, 21.06% LA, 60.99% PA, 1.01% DS and 1.8% SA.
The main causes of waste and percentage over the discard rate were: WB: bad sealed 254(30.56) and clots 238(28.64); RBC: expired 1,087(72) and clots 102(6.75); WB plasma: lipemia 1,066(43.95), Positive Identification Antibody 295(12.16), and RBC contamination 239(9.85); BC: not used 6,211(72) long WB extraction 436(5.05); PP: bad sealed 69(31.36); MC-P: low performance 19(36.53) and RBC contamination 10 (19.23); Ap-PLP: Expired 272(71.76) and underweight 35(9.23).
Summary/Conclusions: Good donor selection, training and evaluation of the staff, implementation of better automatically systems, and analysis of the blood outdated between time intervals will reduce discard of blood components and wastage caused by non conformance improving processes and output of the BTC.

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FRESH FROZEN PLASMA: QUALITY STANDARDS AND CLINICAL EFFICIENCY

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Background: Fresh frozen plasma (FFP) is one of the most frequently used blood component and its use is continuously increasing. Preparation and storage of FFP are strongly defined by European recommendations for the preparation, use and quality assurance of blood components
Aims: The aim of this investigation was to compare the quality of FFP prepared in Blood Transfusion Institute of Niš with current recommendations and to examine the efficiency of FFP transfused to haematological patients.
Methods: FFP separated from whole blood (N = 180) was frozen rapidly at a temperature −75 ± 5°C within 45 min (*Blast shock freezer, Angelantoni, Italy*). After FFP was thawed at 37°C in a water bath, it was investigated for concentration of coagulation factors (FI g/L), FII (%), FV (%), FVII-XII (%)) and inhibitors (AT, PC), all on the ACL Elite Pro IL (*Instrumentation Laboratory, USA*). Clinical efficacy of transfused FFP was evaluated based on post-transfusion INR in the patients (N = 40) four hours after transfusion (INR 1- after 1st transfusion, INR 2- after 2nd transfusion, INR 3- after 3rd transfusion), determination the number of transfused units of FFP per patient and total improvement of INR.
Results: The greatest activity in plasma (%) was recorded for FV, F VII and F XII (128.85 ± 11.95, 126.45 ± 8.03, 123.01 ± 4.58, respectively), while F VIII and F IX showed the lowest activity (FVIII: 103.95 ± 6.66, FIX: 101.75 ± 7.58). The activity of coagulation inhibitors corresponds to the reference values to certain recommendations (a mean value of ATIII was 93.85 ± 4.92%, a mean value of PC was 104.28 ± 8.65%). Investigation of the clinical effectiveness of FFP showed stat
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statistically significant improvement in the measured value of INR with each subsequent transfusion at the level of $P < 0.05$. Comparing the falling value of INR 1 to INR 3, a statistically significant difference was observed between the fall of INR 1 and fall of INR 2 (1.07 vs. 0.79, $t = 2.334$, $P < 0.05$).
Summary/Conclusions: Respecting the basic standards of preparation, as well as adequate storage of FFP units are the basis for optimum product quality. Only high-quality FFP has a satisfactory therapeutic response in patients with clotting disorders.

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QUALITY CONTROL OF BLOOD COMPONENTS IN BLOOD TRANSFUSION CENTRE, FACULTY OF MEDICINE, KHON KAEN UNIVERSITY, THAILAND

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Background: There are Council of Europe (EU), American Association of Blood Banks (AABB) and National Blood Centre, Thai Red Cross Society (TRC) recommendations for the quality of blood components. We analyzed the quality control (QC) of blood components processed by the routine method used in our blood bank.
Aims: To test whether the components reached the recommended quality.
Methods: Packed red cells (PRC),leukocyte poor red cells (LPRC) and leukocyte depleted red cells (LDB) were measured hematocrit, weights and volumes were calculated based on specific gravity. Plasma were measured volumes. Random platelet concentrates (RDP), leukocyte poor platelet concentrates (LPPC) were measured

volumes and platelet content. Cryoprecipitate were measured factor VIII and fibrinogen. For counting residual leukocytes were performed in LPRC, LDB, RDP and LPPC. Sterility test were done by sampling from all types of blood components.
Results: Red blood cell concentrates volume was 150–330 ml (refer to method used), with 50–80% of hematocrit. More than 90% of leukocyte poor red cells had fewer than 1.2×10^9 white blood cells/unit and more than 90% of leukocyte depleted red cells (LDB) had fewer than 1×10^6 white blood cells/unit. Plasma volume was ≥150 ml. Cryoprecipitate showed more than 80 IU/unit of factor VIII and more than 150 IU/unit for fibrinogen. Random platelet concentrates (RDP) had content more than 5.5×10^{10} cells/unit. Leukocyte poor platelet concentrates (LPPC) were more than 24×10^{10} cells/unit and had fewer than 1×10^9 white blood cells contamination. Sterility test were no growth 100%.
Summary/Conclusions: Our QC data of blood components provides reached the recommended quality of Council of Europe (EU), American Association of Blood Banks (AABB) and National Blood Centre, Thai Red Cross Society (TRC).

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Abstract has been withdrawn.

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ASSESSMENT OF QUALITY OF PLATELETS PREPARED BY DIFFERENT PREPARATION PROTOCOLS IN A TERTIARY CARE HOSPITAL OF NORTH INDIA

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Background: Now a days there is need of a platelet product that is more efficacious i.e. good quality with less storage changes, minimal risk of transfusion transmitted infection, low cost and adequate corrected count increment (CCI) after transfusion. There has been an ongoing debate about superiority of one PC type over the other and there are very few studies from this part of the world to address this issue. In this study two types of platelet concentrates (PC), buffy coat pooled (BCP-PC) and single donor apheresis (SDP-PC) were analyzed for quality parameters.
Aims: To assess various markers of platelet quality in different platelet preparation methods.
Methods: A total of 30 PCs (15 BCP-PC and 15 SDP-PC) were included in the study and sampling was done on day 1 and 5. The quality assessment was done for: Volume, Swirling, Platelet count per bag, White Blood Cells (WBC) count per bag, pH changes, Sterility, Mean Platelet Volume (MPV), Platelet Distribution Width (PDW). Independent sample t test was used for comparison.
Results: All the parameters i.e. Mean volume, platelet count, mean WBC count, pH, mean PDW and MPV of BCP-PC when compared with SDP-PC were significantly different. However WBC count was significant on day 1 but non significant on day 5 and pH was non significant on day 1 and significant on day 5. Rest of the parameters were significant on both the days. Grade 3 or 2 swirling was present in all the PCs at any given time of storage and all were sterile.
Summary/Conclusions: In a developing nation like india since apheresis products are expensive and everyone cannot afford it, BCP-PC gives a cheaper alternative with desired clinical benefits. Limitations of our study include lesser sample size, lack of in-vivo measurements like corrected count increment and metabolic changes.

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Abstract has been withdrawn.

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THE PLATELETS PRODUCTION FOR NEONATES IN THE UNIVERSITY HOSPITAL BRNO

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Background: Increasing platelets over 50×10^9/l (in premature children over 100×10^9/l) is a target of platelet transfusions in newborns with thrombocytopenia. There are produced standard pediatric doses of platelets in The University Hospital Brno. It results from dividing standard therapeutic dose for adults into two equal halves. It is recommended to strictly respect AB0 and RhD compatibility of platelets in children. Using AB0 minor incompatible platelets is not accepted because of low weight of neonates. The AB0 antibodies in incompatible platelets can cause hemolysis and makes state of health worse. It is open to question using of platelet additive solutions in neonates. There is this subject still discussed in Czech Republic. There is a fear of higher ion load in platelet additive solutions. The tolerance of newborns to chemicals in additive solutions is lower than in adults and a half-time of elimination is longer. Using platelets resuspended in platelet additive solutions is possible supposing there is not increased risk for children. We started with 100% production of platelets resuspended in PAS in 2010 in The University Hospital Brno.
Aims: The main goal of our research is a verification of PAS safety for newborns.
Methods: The content of ions was calculated in platelets resuspended in PAS and plasma and the results were compared. Adverse events after platelet transfusions in neonates were followed in period 2010–2016.
Results: The natrium citricum content is comparable in both of types platelet products. As for ion content, we founded very low potassium or magnesium levels and we can conclude PAS is safe for neonates. There was reported no adverse event in newborns after platelet transfusion in the period 2010–2016.
Summary/Conclusions: Platelets resuspended in PAS don't mean a higher risk than platelets resuspended in plasma. We registered no adverse event in newborns after platelet transfusion in the followed period.

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QUALITY CONTROL ANALYSIS OF PLATELET CONCENTRATES. IMPORTANT DIFFERENCES BY THE CHOICE OF DEVICE

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Background: Blood counters are usually used to measure peripheral blood counts. In routine quality control measurements of platelet concentrates, counters were also used thereby analyzing very high platelet counts. In order to fulfil requirements of European guidelines, the accuracy of very high platelet counts has to be validated.
Aims: The aim of the present study was to test 5 different blood counter devices focusing on high platelet counts.
Methods: The comparison was performed with platelet concentrates using the blood counter devices CELL-DYN Ruby [A, optical count] and CELL-DYN Emerald [B, impedance count] (Abbott Diagnostics, Wiesbaden, Germany), Sysmex K-4500 [C, impedance count], Sysmex XN-550 [D, impedance count] and Sysmex XN-550 [E, optical count] (Sysmex, Norderstedt, Germany). Platelets were measured at day 1 after collection until day 6 (2 days after shelf life). Furthermore, linear regression analyses were performed using 5 defined concentrations of stock solutions which were serially measured 3 times. For precision performances platelet samples were serially measured 5 times and the coefficients of variation were calculated and compared with manufacturers' declarations. More than 100 blood samples were compared and the normal hemogram parameters (red blood cells, white blood cells, hemoglobin, hematocrit, platelets) were analyzed.
Results: The comparison shows significant differences between the blood counter devices in measuring high platelet counts. The devices B, C, and E measured significant higher platelet counts compared to devices A und D ($P < 0.0001$) independent of the technology of measurement. The manufacturers provide comparable coefficients of variation and linear regressions. We achieved similar results for all counters. All counters showed an expected decrease of platelets at day 6 of storage in a similar manner. All results of the normal blood count parameters were comparable.
Summary/Conclusions: Our study showed the importance of counter validations with the focus of platelet concentrates with a high platelet count before routine use. The manufacturer's peculiarities seem to play an important role, not only the generally fundamental method.

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BLOOD COMPONENT PROCESS OPTIMIZATION, THE RELATION BETWEEN WHOLE BLOOD TEMPERATURE AND PLATELET YIELD

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Background: The preparation of blood components from whole blood vary, including storage time and storage temperature. Different blood components have different storage requirements. Cooling plates have been used to standardize the blood component preparation and the component quality e.g. the factor VIII content. However, a low temperature is considered to have a negative effect on the platelet yield.
Aims: The aims of the study was to evaluate a new system for temperature measurements and furthermore to investigate the temperature effect of the whole blood units in relation to the platelet yield.
Methods: The whole blood bags were put on the cooling plate immediately after donation and stored for a minimum of three hours. The QTA tracer system (Tridentify), a wireless transmitter system, was used to measure the temperature on the cooling plates (butane-1,4-diol plates, Compocool, Fresenius Kabi) and the temperature on the donated whole blood bags. The QTA device is placed on the surface of the blood bag and collects data every three minutes. All data were collected in excel files and temperature curves were analyzed. The cooling plates were pre-cooled in a refrigerator at 4 degrees Celsius according to the manufacturer's instructions. The platelet yield were measured after processing.
Results: In a pilot study the temperature on the cooling plates varied between 11 and 13 degrees Celsius when the first blood unit were placed on the cooling plate.
During storage the blood bags temperature in average dropped in from 26 to 20 degrees Celsius. None of the blood units had a temperature below 20 degrees Celsius at the end of the storage time, with a maximum of 5 h.
The platelet yield in the obtained single platelet units varied between 37 and 101×10^9.
Summary/Conclusions: The QTA tracer system worked smoothly and was easy to handle. The QTA tracer system is a useful tool for process monitoring and quality assurance in the blood component laboratory.
The blood bag temperature was stable and no obvious relation was found between the whole blood bag temperature and the platelet yield. Further studies are in process.

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RED BLOOD CELLS PRE-STORAGE LEUKODEPLETION: QUALITY CONTROLS IN AVERSA BLOOD TRANSFUSION CENTER

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Background: In Italy the DM 02/11/2015 establishes that all the blood components, red cells and platelets, must undergo pre-storage leukocytes reduction by filtration, so as to ensure a residual leukocyte/unit less than 1×10^6, before storing. The only buffy coat removed from the red blood cells reduce the risk of non-hemolytic transfusion reactions because there isn't a leukodepletion $<1 \times 10^6$. The only technique able to reach the expected security is the filtration, that, with the most advanced devices, reduce more than 10,000 times (log 4) the initial leukocyte count.
Aims: The purpose of this study was to perform quality control on blood components to ensure the leukocytes reduction after red cells filtration.
Methods: The pre-storage leukoreduction is performed using the triple top and bottom bags (Macopharma): the whole blood, after 2 h of collection, is centrifugated to 3,250 rpm for 15 min at 22°C with centrifugal Cryofuge 6000i (HERAEUS Instruments); separated in packed red blood cells without buffy coat (EC), buffy coat (BC) and plasma (FP) by COMPOMAT G4 (Fresenius KABI). The EC were then filtered in order to obtain concentrated red cells leukoreduced. The Quality control is performed to evaluate hemoglobin (Hb), red blood cells (RBC) and hematocrit (Hct) using blood count (XT 1800i by Dasit). The residual leukocytes count was performed using a device rWBC ADAM (by Macopharma) that with a specific fluorescent dye for the leukocytes membrane mark and count them.
Results: In the 2016 were produced: 15.072 (79%) concentrated red cells without BC, 1886 (10%) concentrated red cells from apheresis, and 1990 (11%) leukoreduced red cells. In the first two months 2017 were produced 3,200 (96%) pre-storage leukoreduced red cells and 140 (4%) concentrated red cells apheresis. 200 (6%) quality controls were executed. The mean volume of pre-storage leukoreduced red cells was 247 ± 13 ml, the Hb mean was 49 ± 5 g, Hct mean was $59 \pm 4\%$ while the rWBC mean was $0.04 \pm 0.02 \times 10^6$.
Summary/Conclusions: In the 2016, the pre-storage leukoreduced red blood cells production in our Blood Transfusional Center was equal to 21% to reduce costs. Pre-Storage Leukodepletion is also dedicated to transfusion-dependent patients, women of childbearing age and pediatric patients. In 2016 the analysis of 880 concentrated red cells without BC, subjects to quality control, 120 (15%) were discarded for a WBC count outside the limit recommended by the European guidelines (1.2×10^9). No leukoreduced RBCs was discarded for a rWBC count greater than 1×10^6. The aim of the production of blood components is the patient's welfare, this type of blood products in fact could improve considerably the quality of life and transfusion rate, especially in those patients subjected to chronic transfusion therapy.

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Abstract has been withdrawn.

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PREVENTING WRONG COMPONENT TRANSFUSION – IMPORTANCE OF THE FINAL ADMINISTRATION CHECK

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Background: The final administration check must be conducted next to the patient by a trained and competent healthcare professional who also administers the component. Positive patient identification at this final step is essential.
Aims: To determine if the final bedside check, if completed correctly and in full, can detect previous errors and prevent wrong component transfusion (WCT). This includes transfusion of ABO-incompatible red cells.
Methods: A five-year retrospective analysis of cases of WCT (2012–2016). Data collected: the number of cases where an error occurred at or prior to administration, how many administration errors involved a two- or one-person check, whether the wristband was present and correct and if the patient was able to participate in the checking procedure.
Results: WCT could have been prevented in 141/196 (72%) total cases and 26/39 (67%) of ABO-incompatible red cell transfusions, if the administration check had been carried out correctly.
The errors were either at an earlier step in the transfusion process, where the final administration check would have detected 122/196 (62%) of cases or the error occurred at the administration step where a further 19/196 (10%) WCT could have been avoided.
In the majority 90/141(63.8%) the wristband was correct but in 5(3.5%), it was missing (3), or illegible or incorrect (2). (No details given in 46/141(32.6%) cases). Who did the bedside check? In 72 cases this was done by two healthcare professionals, in 33 cases by one (no details given for 36 cases). In 56 cases the patient was able to participate in the bedside check, in 40 cases was unable (45 no details given).
Summary/Conclusions: Despite 5 cases of inadequate wristbands and 40 cases where the patient was unable to participate, the final administration check, if completed correctly, should detect any identification errors and that the component is appropriate and prescribed.
These data demonstrate failures of 2-person checks. Although it is common practice in UK hospitals to have two healthcare professionals perform the final administration check, British Society of Haematology (BSH) Guidelines for administration of blood components state, as a minimum, one professional, must perform the checking/administration procedure, 'Harris et al, BSH, 2009' and that. if local policy requires a two-person checking procedure, each person should complete all the checks independently (double independent checking.) It is essential that every step of the transfusion process each member takes responsibility for their step and not rely on others to get it right.
SHOT recommends use of a 5 point checklist at the patient's side immediately prior to connecting the transfusion 'Bolton-Maggs, SHOT, 2015'. This can act as an aide memoire to complete positive patient identification and the necessary checks of the prescription, wristband and blood component.
It is essential the final administration check is performed in full at the patient's side as this is the final opportunity to get it right and identify any previous errors, will prevent WCT and potentially save lives.

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A COMPARATIVE CLINICAL STUDY OF INTRA ARTICULAR INJECTION OF STEROID, SODIUM HYALURONATE AND PLATELET RICH PLASMA IN OSTEOARTHRITIS OF KNEE

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Background: The prevalence of Osteoarthritis (OA) in India is in the range of 17–60.6%. The treatment is directed as per the Kellgren Lawrence (KL) grading system. Grade 1 is managed with non-pharmacological and non-surgical measures and grade 2 and 3 require intra-articular injections or surgical intervention. The intra-articular injections include platelet rich plasma, local steroids or hyaluronic acid (HA).
Aims: To do a comparative study of intra articular steroid, sodium hyaluronate and platelet rich plasma in moderate OA of knee and compare the results and efficacy.
Methods: This prospective study was conducted at the Department of Transfusion Medicine and Department of Orthopaedics at Indraprastha Apollo hospital, New Delhi in a cohort of 124 patients of KL grade 2 and 3 OA. Forty patients were given intra-articular 40 mg triamcinolone hexacetonate along with 10 ml of 0.25% Bupivacaine. Forty-two patients were given 6 ml (20 mg) of Synvisc (Hylan polymer A and B, G-F 20). Forty-two patients were given PRP 6 ml in each knee. The injections were given after aspiration of excessive synovial fluid. KSS *(Knee Society score)* and VAS *(Visual Analogue Score)* was assessed before injection and at subsequent follow-ups of 1, 4, 12, and 24 weeks. The data was compared within the group using Wilcoxon sign rank test and with one another at different follow-up time using Wilcoxon rank sum test.
Results: The mean age was 52 years and 32% were male and 53% had KL grade 2 OA.
KSS for pain: Baseline score of all the three groups had insignificant differences. Initial large improvement was seen in steroid (from 55.92 before injection to 77.30 at first week and HA (60.14–75.56 at first week,) group compared to PRP (57.83–63.45 at first week). The deterioration of score started after 4 weeks in steroid group (at 24 weeks 61.75) and 12 weeks in HA but it was slow and maintained good result at the end of 24 weeks (76.80). In PRP group the scores improved gradually and the highest score (81.54) was noted at 24 weeks. Trend of improvement of KSS score for function was similar to the pain score.
VAS for Pain: Baseline scores showed a difference between HA and PRP group. Trend of improvement of VAS score was similar to the KSS pain score but only difference seen was in PRP group where the score declined after 12 weeks. Statistically better response from younger patients (age < 50 years) than older one, in all the three groups. When elderly subgroup of HA was compared with elderly subgroup of PRP, better results were seen in patient treated with HA.
Summary/Conclusions: In acute exacerbation of pain, intra-articular steroid relieves pain rapidly and its effect lasts for 8–10 weeks. Effect of intra-articular hyaluronic acid lasts for few months in grade 2 and grade 3 OA. HA was found to be better than PRP in grade 3 OA. PRP is more effective in relatively younger age patients and lower degree of cartilage degeneration and it gives longer duration of pain relief.

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RATES OF ADVERSE REACTIONS ASSOCIATED WITH TRANSFUSION OF BLOOD COMPONENTS AT HOSPITAL LEVEL IN GREECE, 2015

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Background: Adverse reactions (ARs) associated with transfusion are collected at local hospital level in Greece and reported to regional bases of the haemovigilance network. Data analysis is performed by the Coordinating Haemovigilance Centre following national guidelines that are harmonized with Directive 2005/61/EC. The scope of the Greek Haemovigilance System includes evaluating Hospital Quality Indicators and collecting information for monitoring clinical performance as well as efficacy in terms of the outcome of transfusion.
Aims: To analyse rates of transfusion reactions in different hospitals and clinical settings and adverse outcomes from the use of blood components as well as risk factors affecting the quality of the transfused product.
Methods: All transfusion reactions are reported on a standardized form and analysed by type, component, severity and imputability. Data are analysed by different types of hospitals (general, university tertiary, specialized) and diseased category (Haematology, Oncology, Surgery, Cardio Surgery, Orthopedic, Obstetric, General Medicine, Pediatric, Thalassaemia Unit, Others).
Leucodepletion (RBCs with buffy coat, RBC buffy coat removed, RBCs leucocyte depleted pre storage and post storage) irradiation and washing were also examined in relation to adverse transfusion outcomes.
Results: At national level in 2015 we recorded 1570 ARs associated with 768,672 blood components (incidence 1:490). Severe ARs were 119 (1:6.459). Of these, allergic anaphylactic reactions comprised 35%, non- haemolytic febrile reactions 34%, and TACO, TAD and TRALI together 18%. ABO incompatibility due to transfusion of "wrong blood" was diminished in comparison with the previous years. One death was attributed to transfusion, with undetermined cause. In 4/1250 cases, transfusion-associated ARs were known to have serious sequelae.
Detailed data were available from 12 hospitals (7 in Athens/Piraeus, 5 elsewhere) where 199,257 blood components were transfused (46.9% RBCs, 25.5% FFP, 24.3% common platelets and 3.4% units of aphaeresis platelets). Rates of ARs differed significantly between hospitals ($P < 0.001$) ranging from 1:160 to 1:3826.
Among ARs associated with RBCs, 64% were with buffy coat, 8% without, 10% prestorage leucodepletion and 18% post-storage.
Summary/Conclusions: Considerable variation in rates of ARs per hospital was found. Non haemolytic febrile reactions are associated with the administration of non leucodepleted blood components. Transfusion of red cells with buffy coat is associated with two-thirds of all adverse reactions. Therefore pre storage leucodepletion is recommended. Leucodepletion is also recommended for the transfusion of common platelets.

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TRANSFUSION UNDER PRESSURE OF WARMED PLATELET CONCENTRATES VIA A BONE NEEDLE

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Background: In the Erasmus Medical Centre, in case of big traumas often an intraosseous entrance via an Ezio bone needle is combined with a Level 1® H-1025 Fast Flow Fluid Warmer. With this, infusion fluids, including blood products, are administered under pressure. This is done because veins of trauma patients are often not suitable for infusion of fluids. Suppliers of pump and needles describe the possible transfusion of blood products, but this is mainly limited to plasma and erythrocytes. There is no information available concerning transfusion of platelets under pressure via a bone needle.
Aims: To investigate the effects of warming and administration of a platelet concentrate (PC) under pressure via a bone needle on the *in vitro* quality of platelets.
Methods: Pools of 5 BCs and 280 ml of platelet additive solution III (PASIII) were used to make PCs (n = 5). PCs were stored on a flat-bed agitator (60 cycles/min) in a temperature-controlled cabinet at 22 ± 2°C for 4–7 days. To mimic hospital conditions, PCs were warmed using a Fluido® Compact blood warmer and transfused via a bone needle to a transfer bag. On the PCs a pressure of 300 mm Hg was applied. Using clamps, a flow velocity of 90–120 ml/min was realized. Platelet quality before and after pressurized transfusion was determined by means of various *in vitro* parameters.
Results: Due to priming of the transfusion disposable with saline, the PCs were diluted 10–30%, resulting in a significantly increased PC volume and decreased platelet concentration after simulated transfusion. Because of loss of platelets in the disposable set, also the total number of platelets was decreased after simulated transfusion. After simulated transfusion, the PCs still fulfilled the requirements for platelet concentration ($0.8–1.6 \times 10^{11}$/l) and number ($>250 \times 10^{9}$/unit).
Simulated transfusion had no effect on the percentages of CD62P and Annexin V positive cells, indicating no activation or induction of apoptosis due to the simulated

transfusion. pH was not influenced by simulated transfusion. Due to the dilution effect, glucose and lactate concentrations were slightly lower after simulated transfusion.
Summary/Conclusions: Warming and simulated transfusion of PCs under high pressure via a bone needle has no negative effect on the *in vitro* quality parameters of platelets. Transfusion of warmed PCs via an intraosseous entrance via a bone needle is not expected to have a negative effect on the *in vivo* functionality of platelets. It is recommended to study the *in vivo* effects in a limited clinical study.

P-242

Abstract has been withdrawn.

P-243

CLINICAL AUDIT OF THE USE OF FRESH-FROZEN PLASMA IN CARDIOLOGY, CARDIAC AND THORACIC SURGERY PATIENTS

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Background: The appropriate use of blood products allows to manage the transfusion of safe blood products only to treat indicated clinical conditions, leading to significant morbidity or mortality. In the last few years the consumption of fresh-frozen plasma (FFP) in our hospital is significantly decreased, between the various departments, as results of predetermined transfusion guidelines on the clinical use of plasma.
Aims: The aim of this study was to retrospectively evaluate the appropriateness of requests of the FFP using the clinical requests of our blood bank.
Methods: A retrospective analysis of the FFP requests from departments of cardiology,cardiac and thoracic surgery, during a 12 month period between January 2016 and December 2016 was executed. It was collected the follow clinical information: personal data, diagnosis, indication for the use of FFP (DIC, warfarin overdose, massive transfusion, etc.), laboratory data (PT, aPTT, INR, fibrinogen), the amount of the required FFP, transfused quantity of FFP.
Results: We analyzed a total of 1,314 requests. The amount of required FFP was 6,096 units, which 3,032 was provided between the various clinical departments. The provided FFP units was selected in this way: 1,993 plasma safe, 320 by apheresis, 719 FFP by whole blood. Overall, only 50.3% of the FFP analyzed requests were considered appropriate and were for appropriate indications. As for FFP usage in common clinical indications, there was a high incidence of inappropriate use in perioperative period (55%), cardiac surgery (52%), massive bleeding (71%) and trauma (50%).
Summary/Conclusions: In order to minimize the transfusion risks for the patients is important a critical approach to the use of the FFP. For this reason, we have planned for the 2017 year a hospital training in order to evaluate the appropriateness of all of the FFP request by a prospective evaluation of the same. On the other hand, the assessment of appropriateness is an essential step of the FFP management, in order to achieve the dual purpose of improving the quality and reduce the health costs.

P-244

OXYGEN CONTENT–UNCONTROLLED AND OVERLOOKED PARAMETER ASSOCIATED WITH STORED RED CELL CONCENTRATE QUALITY: UNEXPECTEDLY WIDE DISTRIBUTION

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Background: Oxygen is the main substrate for oxidative reactions and the resulting oxidative damage is considered one of the major causative factors of the development of the red blood cell (RBC) storage lesion. Oxygen saturation (SO_2) of venous blood is generally assumed to be around 70–80% as measured from a central venous line. However, a recent investigation of SO_2 levels in freshly procured red cell concentrates (RCC) revealed unexpectedly wide distribution of SO_2 (mean 45.9 ± 17.5%). Previous studies showed significant negative consequences of high SO_2 levels on the quality of RBC during hypothermic storage [Yoshida et al 2017 Blood Transfusion].
Aims: The main objective of the present study was to determine the SO_2 distribution in leukoreduced RCC produced at a medium-size blood center using a novel non-invasive probe.
Methods: The following room temperature component processing methods were used to prepare 977 units of leukoreduced RCC at Rhode Island Blood Center (Providence, RI, USA) on January 9–13 2017, representing 78% of collection volume; platelet-rich plasma (48.1%; Haemonetics 123-63/RCM1 filter), cryoprecipitate (33.4%; Haemonetics 129-63/RC2D) and apheresis double RBC (18.5%; Haemonetics 832F/RC2D), all processed within 8 h from blood collection time. SO_2 was measured non-invasively through the PVC bag immediately prior to storage by a Resonance Raman Spectroscopy (Pendar Microvascular Oximeter A3U11; Pendar Technologies, Cambridge MA, USA). Process method, RCC volume, blood type, gender and process time were also recorded.
Results: Non-invasive SO_2 measurements at the blood center showed a similar broad distribution as a previous study of 497 procured RCC units (within 24 h of blood collection) using invasive sampling for SO_2 determination with cooximetry [Yoshida 2017 ibid]. Overall, the shape of the SO_2 distribution appeared near normal with the mean of 47.0%±21.0%; median 45.2%; range <5% to >95%; and interquartile range (IQR) of 31.4%>61.9%. Apheresis RCC showed significantly higher SO_2 ($P < 0.05$) [mean 56.7%±16.1%; median 55.6%; range 24% to >95%; IQR 47.0%>67.0%] compared to RCC prepared rom the platelet-rich plasma procedure [mean 43.9%±20.7%; median 43.2%; range <5% to >95%; IQR 27.5%>59.0%] or the cryoprecipitate procedure [mean 46.2%±22.2%; median 42.7%; range <5% to >95%; IQR 29.5%>59.9%]. Male donors showed higher SO_2 compared to female donors ($P < 0.04$) even when excluding apheresis RCC units which are from predominantly male donors [Female—mean 42.7%±21.0%; median 39.3%; range <5% to >95%; IQR 27.5%>57.0%], [Male—mean 47.2%±21.0%; median 45.2%; range <5% to >95%; IQR 30.7%>61.9%]. No correlations were observed between SO_2 levels and processing time, donor age or blood types
Summary/Conclusions: The surprisingly disperse distribution of starting SO_2 observed in this study most likely originated from individual donors, as addition of air or extensive oxygen consumption during component processing cannot account for units deviating by more than 40% SO_2 from the median value. Considering recent reports showing profound effects of SO_2 levels on RBC metabolism, coupled with the negative impacts of high SO_2 on the quality of stored RBC suggests that oxygen levels are an important and underappreciated source of unit-to-unit variability in RBC quality.

Plasma products

P-245

Abstract has been withdrawn.

P-246

Abstract has been withdrawn.

P-247

CHROMATOGRAPHY AS AN ESSENTIAL TOOL IN THE MANUFACTURE OF PLASMA PRODUCTS

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Background: Plasma-derived medicinal products (PDMPs) are valuable class of therapeutics, used for prevention, management and treatment of coagulation factor deficiencies, metabolic and thrombotic disorders, immunological diseases, infections and several other life-threatening conditions.
Aims: Today, the industrial scale manufacturing of these products has evolved from those primarily based on traditional backbone of cold ethanol fractionation to integrated hybrid processes including cryoprecipitation and ethanol fractionation, chromatographic procedures and pathogen reduction technologies.

Methods: Different chromatographic techniques including Ion Exchange Chromatography, Affinity Chromatography and Size Exclusion Chromatography, are widely used alone or in combination with each other in various industries. Among these industries, chromatography has brought a significant impact in the plasma fractionation industry by increasing the quality, purity, safety, yields, and diversity of plasma products as well as improving the cost-effectiveness of production method. In addition in most Pharmacopoeias, chromatographic methods are widely used as the only valid method for the analysis of plasma products.
Results: Therefore implementation of selective and specific chromatographic techniques is of particular value in extraction, isolation, purification, manufacture and analysis of PDMPs. Moreover, their crucial role should not be forgotten in the removal of plasma-borne viruses, unwanted chemicals and protein contaminants.
Summary/Conclusions: Considering the growing demand for PDMPs, it seems that the role of chromatography in the manufacture of plasma products become more significant in the future.

Pathogen inactivation

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QUALITY OF RED BLOOD CELLS DERIVED FROM WHOLE BLOOD TREATED WITH UV LIGHT AND RIBOFLAVIN

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Background: Pathogen reduction technology (PRT) increases blood transfusion safety by reducing the risk of transmitting pathogens and white blood cells. Despite implementation of pathogen testing and donor screening, a residual risk remains due to the window period between infection and detectable pathogen load. Furthermore, emergent, not-yet-tested-for blood-borne pathogens can be a threat to patients and blood supply, as exemplified by the recent Zika outbreak. The Mirasol® PRT System uses riboflavin (vitamin B2) and UV-light to alter nucleic acids and inhibit replication of pathogens and white blood cells. Initially developed for platelets and plasma treatment, the Mirasol PRT System is now the only PRT that is also CE-marked for treatment of WB. Red blood cells derived from Mirasol-treated WB are under development as an investigational product in the United States.
Aims: To assess the quality of RBCs derived from WB treated with the Mirasol PRT System.
Methods: Paired WB units from 61 healthy donors were collected in CPD on 2 occasions ≥8 weeks apart. One unit from each donor was treated with the Mirasol PRT System (riboflavin + UV light 80 J/ml_{RBC}) and the other was used as untreated control. RBCs were processed from the WB units, leucoreduced and stored in AS-3 at 1–6°C for 21 days and sampled for in vitro analyses of RBC quality parameters, including, for a subset, oxygen dissociation curves (ODCs). To explore the potential of using storage solutions other than AS-3 for RBCs derived from Mirasol–treated WB, 2 separate research studies were performed evaluating the degree of haemolysis when using 1) AS-3, SAGM and PAGGSM for RBCs processed using the buffy coat method and 2) the anticoagulation/storage solution combinations CPD/AS-1, CP2D/AS-3, and CPD/AS-5 for RBCs by the platelet-rich-plasma method.
Results: RBCs derived from Mirasol-treated WB and stored in AS-3 met FDA criteria of haemolysis (<1%) and pH (>6.2) in all units after 21 days of storage. Notable differences between Mirasol-treated units and control units were extracellular potassium concentrations ($[K^+]$) (68.4 ± 4.1 vs 37.3 ± 4.6 mmol/l after 21 days) and methaemoglobin (4.9 ± 2.2% post-treatment vs 0.6 ± 0.4% post-collection). A separate sub-study showed that $[K^+]$ in Mirasol-treated RBCs was slightly higher than in gamma irradiated RBCs, and that methaemoglobin returned to post-collection values within 24 h. Mirasol treatment shifted ODCs slightly left but did not alter their shape. The ATP concentration of Mirasol-treated RBCs after 21 days (4.9 ± 0.9 μmol/gHb) was similar to concentrations previously shown to correlate with adequate RBC recovery in vivo (Cancelas, Transfusion, 2017). Mirasol-treated RBCs stored in AS-3, PAGGSM and SAGM met Council of Europe guidelines of <0.8% haemolysis in all units through 21 days at 1–6°C. Mirasol-treated RBCs stored in CPD/AS-1, CP2D/AS-3, and CPD/AS-5 maintained <1% haemolysis in all units through day 21, 21, and 14, respectively.
Summary/Conclusions: RBCs derived from WB collected in CPD and treated with the Mirasol System maintained adequate in vitro properties for the US market when stored in AS-3 in 1–6°C for 21 days. Storage in other additive solutions maintained low haemolysis through 21 days, showing promise for potential future use in other markets.

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QUALITY OF WHOLE BLOOD TREATED WITH UV LIGHT AND RIBOFLAVIN AFTER 24 H IN ROOM TEMPERATURE

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Background: Transfusion of non-componentized whole blood (WB) is the norm in far-forward combat zones and in many developing countries, often coinciding with an increased risk of transfusion-transmitted infections due to limited pathogen screening and high pathogen exposure. Pathogen reduction technology (PRT) can increase blood safety, but has until recently only been available for componentized platelets and plasma. The Mirasol® PRT System for WB, which was CE marked for WB in 2015, is a non-toxic, non-mutagenic PRT that uses riboflavin (vitamin B2) and UV light energy to alter nucleic acids, thereby inhibiting replication of pathogens and white blood cells. The clinical trial African Investigation of Mirasol System for Whole Blood (AIMS) showed that Mirasol treatment of WB reduced the incidence of transfusion-transmitted malaria. Use of PRT-treated WB in austere and/or resource-scarce environments may involve sub-optimal handling such as delayed refrigeration and delayed PRT treatment.
Aims: To assess the quality of WB treated with the Mirasol PRT System when Mirasol treatment is performed after 24 h in room temperature (RT) and then stored at 1–6°C.
Methods: Fourteen units of 500 ± 50 ml WB collected in 70 ml citrate phosphate dextrose anticoagulant (CPD) were held at RT for 24–28 h post-collection before Mirasol treatment (exposure to UV energy [80 J/ml_{RBC}] in the presence of 35 ml of 500 μM riboflavin) and storage at 1–6°C for 21 days. Units were sampled for in vitro analyses of WB quality parameters (complete blood count, blood gases and electrolytes), platelet assays (P-selectin and Annexin V) and plasma assays (prothrombin time [PT], activated partial thromboplastin time [APTT], Factor VIII, fibrinogen).
Results: All units met Council of Europe (COE) acceptance criteria of hemolysis (< 0.8 %) and pH (> 6.2) through 14 days of storage. After 21 days, 4 units exceeded hemolysis of 0.8%. Mean extracellular potassium levels increased from 4.1 ± 0.3 mmol/l pre-treatment to 41.2 ± 3.5 mmol/l at day 21. Mean factor VIII and fibrinogen retention directly after Mirasol treatment was 79 ± 12% and 81 ± 8%, respectively. The PT and APTT clotting times increased on average 1.3 ± 0.6 s and 5.8 ± 2.0 s, respectively, as a result of treatment and by 1.6 ± 1.1 s and 2.5 ± 1.7 s, respectively, as a result of 7 days storage. ROTEM analysis at day 7 showed functioning coagulation, albeit with a decrease in clot formation quality.
Summary/Conclusions: Whole blood treated with the Mirasol PRT System for WB after 24–28 h in RT and stored refrigerated for 14 days meets COE criteria of hemolysis < 0.8 % and pH > 6.2. When WB is Mirasol-treated within 8 h of collection, these criteria are met for 21 days (Owusu-Ofori, Shock, 2014). The need for added flexibility in the timing of treatment may sometimes exceed the need for longer shelf life, and an increase in clotting time may be an acceptable trade-off for obtaining pathogen reduced WB in settings where the risk of transfusion-transmitted infections is high.

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CHARACTERIZATION OF INTERCEPT BLOOD SYSTEM FOR RED BLOOD CELLS USING SAGM RBCS PREPARED USING MANUAL AND AUTOMATED WHOLE BLOOD SEPARATION TECHNIQUES

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Background: The INTERCEPT™ Blood System for Red Blood Cells (RBCs) inactivates pathogens and leukocytes in RBC components for transfusion using amustaline to form adducts with nucleic acids, preventing replication of contaminating pathogens and leukocytes. A Phase 3 clinical investigation in patients with thalassemia major requiring chronic RBC transfusion support is in progress in Europe. Ege University Hospital is providing study RBCs to evaluate the safety and efficacy of INTERCEPT RBCs compared to conventional RBCs.
Aims: This study was designed to qualify Ege University Hospital Blood Bank to produce INTERCEPT RBCs for use during the Phase 3 clinical investigation with SAGM RBC prepared using manual or automated whole blood separation.

Methods: On the day (D) of collection, D0, CPD whole blood was processed using manual (M) or automated (A; Reveos system, Terumo BCT, USA) methodologies. SAG-M RBCs were stored at 4 ± 2°C until treatment on D1 with the INTERCEPT process.
Leukocyte depleted SAG-M RBCs (Test-M: 226–286 ml and Test-A: 260–321 ml) were added to processing solution containing glutathione (GSH) followed by amustaline addition (final concentrations of 20 mM GSH/0.2 mM amustaline, based on 280 ml RBC input). After 18–24 h hold at 20–25°C, RBCs were centrifuged and the supernatant was replaced with SAG-M. INTERCEPT RBCs were stored at 4 ± 2°C for 35 days and were sampled on D2, D14 and D35 for analysis of *in vitro* physical and metabolic parameters.
Results: All units met the acceptance criteria for site qualification. The volume post treatment was 226–295 ml (Test-M) and 250–337 ml (Test-A), with a loss of 3 ± 2 g of Hb attributed to the INTERCEPT process. All units had Hb values of ≥40 g ranging from 41–58 g. The final Hct was 55–63%, within the 50–70% criterion. After 35 days of storage all INTERCEPT RBCs met the acceptance criteria of ≤0.8% hemolysis; hemolysis was higher in Test-M (0.45 ± 0.18%) compared to Test-A components (0.18 ± 0.06%). ATP values exceeded 2 μmol/g Hb.
Summary/Conclusions: The INTERCEPT Blood System for RBC technology was successfully validated at Ege Blood Bank. This study demonstrated that SAG-M RBC inputs prepared using manual and automated WB separation methods are compatible with the INTERCEPT Blood System for RBC. INTERCEPT RBC units met the EDQM guidelines (18th Ed.) for leukocyte depleted RBCs in additive solution with respect to Hct, Hb content and hemolysis at end of storage. All measured *in vitro* parameters of INTERCEPT treated RBCs, including ATP levels, indicate suitability for transfusion.
INTERCEPT Blood System for Red Blood Cells is not approved for commercial use.

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EFFECTIVENESS OF THE THERAFLEX UV-PLATELETS TECHNOLOGY AGAINST CLINICALLY RELEVANT TRANSFUSION-TRANSMITTED BACTERIA STRAINS

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Background: The THERAFLEX UV-Platelets system (Macopharma) is a late generation pathogen inactivation system for platelet concentrates (PCs) that uses UVC light only (wavelength: 254 nm) without the need of any additional photoactive compound. UVC treatment has been shown to inactivate a broad range of viruses, bacteria, and protozoans. Previous studies with the first set of bacteria species of the WHO International Repository of Platelet-Transfusion Relevant Bacterial Reference Strains revealed a high inactivation capacity for clinically relevant bacteria.
Aims: Aim of the current study was to validate the bacteria inactivation capacity using bacteria species that have recently been added to the WHO International Repository of Platelet-Transfusion Relevant Bacterial Reference Strains.
Methods: PCs were produced from 5 buffy coats using the additive solution SSP+ (MacoPharma) with a residual plasma content of 35%. For inactivation kinetics, PCs (n = 3) were spiked with bacteria to a final concentration of approx. 10^6 colony forming units (CFU)/mL and irradiated with increasing doses until the full dose was UVC was achieved. Samples were taken for the bacterial titer determination after each irradiation step.
Results: Treatment with the THERAFLEX UV-Platelets pathogen inactivation system caused dose-dependent inactivation of bacteria in plasma-reduced PCs. Mean $\log_{10}$ reduction factors ranged from 6 to 7 for Enterobacter cloacae (PEI-B-P-43), Morganella morganii (PEI-B-P-91), Proteus mirabilis (PEI-B-P-91), Pseudomonas fluorescens (PEI-B-P-77), Staphylococcus aureus (PEI-B-P-63), and Streptococcus bovis (PEI-B-P-61).
Summary/Conclusions: The THERAFLEX UV-Platelets system efficiently inactivates a broad range of different bacteria species, including the WHO reference strains.

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INACTIVATION OF BIOFILM-DERIVED STAPHYLOCOCCUS EPIDERMIDIS IN PLATELET CONCENTRATES WITH RIBOFLAVIN AND ULTRAVIOLET LIGHT TREATMENT

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Background: The predominant aerobic bacterial contaminant of platelet concentrates is *Staphylococcus epidermidis*. This bacterium forms surface-attached aggregates (biofilms) in platelet concentrates, which could increase missed detection during routine platelet screening. The Mirasol Pathogen Inactivation system uses riboflavin as a photosensitizer which modifies nucleic acids by causing irreversible changes upon exposure to ultraviolet light (UV) resulting in inactivation of a broad spectrum of Gram positive and Gram negative bacteria. No investigations have been performed to evaluate Mirasol inactivation of bacterial biofilms.
Aims: This study was aimed at evaluating the efficacy of riboflavin-UV treatment to inactivate biofilm-derived *S. epidermidis* in buffy coat platelet pools.
Methods: Biofilm and non-biofilm cells from *S. epidermidis* ST-10002 (platelet contaminant) and *S. epidermidis* AZ-66 (skin isolate) were individually inoculated into whole blood units at a concentration of ~10^6 colony forming units per millilitre (CFU/mL) (N = 4–5). One spiked and three unspiked whole blood units were processed to produce one buffy coat platelet pool. Riboflavin was added to the pool, which was split into two bags: one for UV treatment and the second was left untreated as a control. Bacterial loads were measured before and after treatment. Changes in the *in vitro* quality of the platelet pools were assessed by flow cytometry (CD62 expression as response to ADP) and dynamic light scattering (ThromboLUX device).
Results: Bacterial counts were reduced during buffy coat platelet production from ~10^6 CFU/ml in whole blood to 10^3–10^4 CFU/ml in the pools ($P < 0.0001$). Riboflavin-UV treatment resulted in significantly higher reduction of *S. epidermidis* AZ-66 than *S. epidermidis* ST-10,002 (≥3.5 log reduction and 2.6–2.8 log reduction, respectively, $P < 0.0001$). No differences in *S. epidermidis* inactivation were observed in platelet pools produced from whole blood inoculated with biofilm or non-biofilm cells ($P > 0.05$). Interestingly, platelet activation was enhanced in pools produced with whole blood units inoculated with biofilm cells compared to non-biofilm cells ($P < 0.05$).
Summary/Conclusions: The efficacy of riboflavin-UV treatment was similar in platelet pools produced from whole blood inoculated with *S. epidermidis* biofilm or non-biofilm cells. Platelet activation in pools derived from whole blood inoculated with biofilms may be due to increased interactions between platelets and biofilm aggregates. Levels of biofilm-derived *S. epidermidis* ≥10^3 CFU/ml were not completely inactivated by riboflavin-UV; however, further testing is necessary with lower (real-life) bacterial loads and UV illumination at different times.

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IN VITRO ASSESSMENT OF UNTREATED, UVC-TREATED AND GAMMA-IRRADIATED PLASMA REDUCED PLATELET CONCENTRATES PREPARED FROM THROMBAPHERESIS UNDER ROUTINE CONDITIONS

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Background: Pathogen reduction technology may enhance microbial safety of platelet transfusion by reducing bacterial and viral contamination. We established the manufacturing of UVC-treated apheresis platelet concentrates (PCs) under routine conditions.
Aims: The objective of this study was to evaluate potential *in vitro* effects of the THERAFLEX UV-Platelets treatment on apheresis platelets in comparison to untreated and gamma irradiated apheresis platelets.
Methods: Twenty-four leukocyte reduced and plasma reduced platelet concentrates (PCs) were prepared by apheresis using SSP+ as additive solution (Macopharma, Mouvaux, France).
We established the preparation of three different platelet products: PCs treated with the THERAFLEX UV-Platelets system (Macopharma) within 6 h after PC preparation,

untreated PCs and gamma-irradiated (30 Gy) PCs. Platelet products were all stored for 7 days in the storage bag of the THERAFLEX UV-Platelets kit.
To evaluate the quality of these products, we analyzed product volume, residual erythrocytes, residual leukocytes, platelet content, total protein concentration, pH, sterility and CD62P expression. CD62P surface expression, a marker of platelet activation, was analyzed by flow cytometry with and without activation by thrombin-receptor activating peptide (TRAP) after production and at the end of shelf life. For statistical analysis the Kruskal-Wallis-test was applied, and P-values <0.05 were considered as statistically significant.
Results: UVC-treated PCs showed no significant differences compared to untreated or gamma-irradiated PCs with respect to volume (untreated PCs 330 ± 11 ml, gamma irradiated PCs 328 ± 5 ml, UVC-treated PCs 328 ± 13 ml), platelet content (untreated PCs $2.9 \pm 0.3 \times 10^{11}$ per unit, gamma irradiated PCs $2.9 \pm 0.2 \times 10^{11}$ per unit, UVC-treated PCs $2.9 \pm 0.1 \times 10^{11}$ per unit), residual leukocytes, residual erythrocytes, total protein concentration (untreated PCs 23 ± 1.5 g/l, gamma irradiated PCs 23 ± 1.8 g/l, UVC-treated PCs 23 ± 1.9 g/l) and pH at the end of shelf life.
CD62P expression was variable between the PC groups with and without activation by TRAP but did not reach statistical significance.
Tests for bacterial contamination were negative for all tested PCs.
Summary/Conclusions: This data indicate that plasma reduced, UVC treated PCs meet the quality standards for PC products according to the German Guidelines. Safety, tolerance and efficacy of PCs manufactured by the THERAFLEX-UV Platelets system are currently evaluated in a clinical study.

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RED BLOOD CELL CONCENTRATES TREATED WITH THE AMUSTALINE (S-303) PATHOGEN REDUCTION SYSTEM RETAIN ERYTHROCYTE MORPHOLOGY AND METABOLIC PROPERTIES

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Background: Red blood cell concentrates (RBCC) are the most widely transfused blood component. A protocol that reconciles long-term storage and safety of the therapeutic component is still a subject of debate. Storage of RBCC at 4 °C in storage medium is associated with metabolic, morphologic and biochemical changes, collectively referred to as to "storage lesions". The pathogen inactivation INTERCEPT Blood System for red blood cells utilizes amustaline (S-303) to inactivate a broad range of pathogens in RBCC. Amustaline is a nucleic acid-targeted alkylator that inactivates contaminating viruses, bacteria, parasites and leukocytes and is designed to react quickly and to degrade to a non-reactive species.
Aims: The objective of this study is to evaluate the effects of pathogen inactivation on RBCC functionality and morphology throughout the whole 42-days storage period.
Methods: Leukoreduced RBCC are treated or not with amustaline (4 control RBCC, 4 Intercept-RBCC) and stored at 4°C for 42 days. The experiments are conducted blindly. The morphology, hematocrit, hemoglobin content, residual leukocytes count, hemolysis and ATP levels are investigated at different days of storage. The cell shapes are observed using scanning electron microscopy (SEM) and cells are classified in 3 groups following Bessis' classification (*Nouv. Rev. Fr. Hematol., 1972).* The percentages of i) discocytes, ii) echinocytes and stomatocytes (reversible membrane-shape alteration) and iii) spherocytes (irreversible alteration) are evaluated by counting about 600 cells in 5 randomly chosen fields.
Results: The mean values of *in vitro* parameters for Intercept-RBCC meet the EU guidelines for leukocyte depleted RBCC: volume >225 ml, hemoglobin content >40 g/RBC unit, hematocrit 50–70, and residual leukocyte content $<1 \times 10^6$/RBC unit. ATP levels of Intercept-RBCC, throughout the storage duration do not exceed 2 μmol/g hemoglobin, the criteria for acceptable viability of RBCC. Hemolysis increases weakly in a time dependent manner (0.1–0.4%), similarly in both control and Intercept treated RBCC. SEM observation indicates an alteration of the RBC morphology. The number of RBC displaying irreversible shape changes increases at day 10 and reaches 17% at days 42 in both groups. This suggests that the time dependent morphological changes is occurring throughout storage in a comparable manner in both control and Intercept RBCC.
Summary/Conclusions: The morphology and *in vitro* parameters of Intercept RBCC indicate their suitability for transfusion and reveal no differences with respect to control RBCC even after 42 days of storage. These results are in accordance with the studies of Cancelas *et al* (*Vox Sanguinis 2017)* showing that the inactivation process is metabolically and physiologically appropriate for transfusion.

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STORAGE STUDY OF APHERESIS PLATELETS IN ADDITIVE SOLUTION AFTER PHOTOCHEMICAL TREATMENT USING A NOVEL TRIPLE STORAGE SET

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Background: Photochemical treatment is intended to prevent transfusion-transmitted infections by inactivating pathogens in platelet concentrates (PC). A novel triple-storage set was developed for photochemical treatment of double or triple PC re-suspended in plasma/Platelet Additive Solution (PAS) ($5–12 \times 10^{11}$ platelets in 420–650 ml).
Aims: This study evaluated *in vitro* function after INTERCEPT™ treatment (Cerus) of platelets collected with two different apheresis collection devices using a set with 3 storage containers (TS) compared to a set with single storage container (LV) over 7 days-storage.
Methods: After collection on Trima Accel® (Terumo BCT) or Amicus® (Fresenius Kabi) separator, one single and one double PC were pooled in respect of separator origin. This pool was split back (2/3 and 1/3 volumes) to generate units compatible for photochemical treatment with respectively TS (Test) and LV (Control) INTERCEPT™ sets. Test units contained about 8×10^{11} platelets/PC and control units 4.5×10^{11} platelets/PC, both in 62% PAS. Each test unit (580 ml) was connected to a TS sets for addition of amotosalen (S-59), UVA illumination and transferred to a double Compound Adsorption Device (CAD) for agitated storage for 4 h, and finally split into 3 identical PC. Similar process was applied to the Control units (320 ml) using LV set and 6 h single CAD treatment. Platelet quality was evaluated in a storage study on Day 1, 3, 5, and 7 after donation by measuring ATP, glucose, sCD62p, LDH, RANTES and sCD40L concentration. All results were normalized for platelet content prior to statistical analyses (paired and unpaired Student's t-test).
Results: Day 1 volume was 182 ± 14 ml in Test *vs* 304 ± 10 ml in Control units. Platelet content was $2.55 \pm 0.22 \times 10^{11}$ in Test vs $4.10 \pm 0.43 \times 10^{11}$ in Control units. Platelets in both sets showed a normal metabolism with moderate consumption of ATP. There was no statistically significant difference for glucose concentration in the storage study for the LV set compared with TS set. However, the difference was statistically significant between the two apheresis systems Amicus® and Trima Accel®. Glucose reserves were exhausted at day 7 for units collected with Amicus® separator. There was a moderated increase of sCD62p and LDH during storage ($P < 0.05$ between day 1 and day 7). We observed no statistically significant difference for sCD62p and LDH between Test and Control units. RANTES and sCD40L increased with storage in both arms ($P < 0.05$ between day 1 and day 7) but results are in an acceptable range and the difference between Test and Control group was not statistically significant. However, statistical significant differences were observed between the two devices for LDH, sCD62p and sCD40L. Residual amotosalen was <0.5 μmol/l in both Control and Test units.
Summary/Conclusions: The storage study of apheresis platelet demonstrates no significant difference between TS and LV sets for up to 7 days of storage. Differences between Amicus Trima Accel® and ® PC sets, independent of INTERCEPT™, were observed. These differences will be further investigated.

P-256

Abstract has been withdrawn.

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INFLUENCE OF THE TEMPERATURE ON THE QUALITY AND VIRUS INACTIVATION CAPACITY OF METHYLENE-BLUE TREATED PLASMA USING THE THERAFLEX MB-PLASMA SYSTEM

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Background: Photodynamic treatment using methylene blue (MB) and visible light is in routine use for pathogen reduction of human plasma in different countries. Temperature conditions of either environment or human plasma during production might vary between production sites.

Aims: Aim of the study was to investigate the influence of different temperature conditions on the quality and virus inactivation capacity of the THERAFLEX MB-plasma procedure (MacoPharma).
Methods: For quality investigations, plasma (n = 3) was pooled and split and either stored at room temperature (reference) or stored at 5 °C and 30 °C (test) for a minimum of 2 h to ensure equilibration to the desired temperature. Plasma unit (n = 8 for each temperature) was connected to the MB bag system including a leukocyte depletion filter (Plasmaflex) and a filter for the removal of MB and its photoproducts (Blueflex). Treatment was done according to the manufacturer's instructions on the Macotronic B2 device (MacoPharma) with a light dose of 120 J/cm^2. Samples were taken at different process steps to determine the activity of plasma factors and the concentrations of MB and photoproducts. For virus inactivation kinetics, plasma units (n = 3 for each temperature and virus species) were spiked with virus suspension (10%) of three different virus species and MB/light treated. Samples were taken after different dose steps and virus titres were determined by endpoint titration.
Results: MB/light treatment affected the activity of most of the investigated coagulation proteins and inhibitors. The highest decreases in reference conditions were detected for Factor VIII (−17.7%, 22 °C) and Fibrinogen (−14.4%, 22 °C). There was a trend towards a higher loss of plasma factor activities with increasing plasma temperatures, albeit differences were statistically not significant, except for Factor VIII (P = 0.044). However, plasma temperature significantly influenced the solubility of the MB pill, the concentrations of MB and photoproducts after illumination and the virus inactivation capacity. Solubility of the MB pill was visibly reduced at 5 °C. Degradation of MB during illumination increased with increasing temperature, resulting in augmented formation of photoproducts (mainly Azure B) at 30 °C. However, the filtration efficacy of the Blueflex filter was sufficient to remove the photoactive compounds. Virus inactivation capacity increased with plasma temperature rising. Inactivation of Suid Herpes Virus (SHV-1), Bovine Viral Diarrhoea Virus (BVDV) and Vesicular Stomatitis Virus (VSV) was significantly lower for plasma units with a temperature of 5 °C than for plasma with temperatures of 22 °C and 30 °C.
Summary/Conclusions: The conditions for THERAFLEX MB-plasma treatment in routine production have to be chosen carefully to assure uniform quality of the pathogen-reduced plasma. Inactivation of cooled plasma is not recommended.

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ZIKA VIRUS INFECTIVITY IS REDUCED FOLLOWING TREATMENT WITH THE THERAFLEX UVC-PLATELET AND THERAFLEX MB-PLASMA SYSTEMS

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Background: Zika virus (ZIKV) has emerged as a pathogen of global significance with evidence of mosquito-borne transmission in 76 countries and territories since 2007. Given that asymptomatic infection can occur and that transfusion-transmission has been documented, this virus poses a potential threat to blood transfusion safety. In Australia there has been no local ZIKV transmission to date; however, vectorial capacity modelling suggests such a scenario is possible in areas with the mosquito vector. The Australian Red Cross Blood Service prevents individuals with a symptomatic illness or confirmed ZIKV infection from donating blood, and restricts fresh component donation from individuals returning from travel to countries with autochthonous ZIKV transmission. An alternative approach to manage the ZIKV transfusion-transmission risk is the use of pathogen inactivation (PI) systems, such as THERAFLEX UV-Platelet and THERAFLEX MB-Plasma.
Aims: To investigate the efficacy of the THERAFLEX UV-Platelet and the THERAFLEX MB-Plasma systems to inactivate ZIKV spiked into buffy coat-derived platelet concentrates (PCs) or plasma.
Methods: ZIKV was spiked into PCs or plasma units (n = 3 per blood component) to give a pre-treatment concentration of 10^5 – 10^6 plaque forming units per mL. Spiked PCs were treated using the THERAFLEX UV-Platelet system at UVC doses of 0.05, 0.10, 0.15 and 0.20 (standard) J/cm^2. Spiked plasma units were treated using the THERAFLEX MB-Plasma system at a visible light doses of 20, 40, 60 and 120 (standard) J/cm^2 in presence of approximately 0.8 μmol/l of methylene blue (MB). Samples were taken prior to the first and after each illumination dose and tested for residual virus using a modified plaque assay (normal and large-volume plating methods). For each PI system the level of viral reduction was determined.
Results: Treatment of PCs with THERAFLEX UV-Platelet system resulted in an average of 5 $\log_{10}$ reduction in ZIKV infectivity at the standard UVC dose (0.20 J/cm²), with dose-dependency observed with increasing UVC dose. For pooled plasma treated with MB and visible light, ZIKV infectivity was reduced by an average of at least 5.68 $\log_{10}$, with residual viral infectivity reaching the detection limit of the assay at 40 J/cm^2.
Summary/Conclusions: This study has demonstrated that the THERAFLEX UV-Platelet and the THERAFLEX MB-Plasma systems can inactivate ZIKV spiked into PCs or plasma. The level of reduction observed (average of 5 $\log_{10}$ in PCs and average of at least 5.68 $\log_{10}$ in plasma) with these PI systems is similar to that for other viruses, including dengue, chikungunya and West Nile viruses. To assess if the level of reduction in viral infectivity in these blood components is sufficient to prevent transfusion-transmission, studies examining the threshold concentration to elicit disease are needed. However, the reduction levels observed in this study demonstrate that these PI systems could be an effective option for managing ZIKV transfusion-transmission risk in plasma and PCs.

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EVALUATION OF APHERESIS PLATELETS PROCESSED WITH THE INTERCEPT BLOOD SYSTEM FOR PLATELETS TRIPLE STORAGE SET

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Background: The INTERCEPT™ Blood System for platelets is intended for the *ex vivo* preparation of pathogen-inactivated apheresis or whole blood-derived platelet components (PCs) in order to reduce the risk of transfusion-transmitted infection and transfusion-associated graft vs host disease. Platelets suspended in platelet additive solution (PAS) can be treated with any of the three licensed INTERCEPT Platelet Processing Set configurations for platelet doses of 2.5 to 8.0 × 10^{11} platelets. Cerus has designed a platelet processing set with three storage containers to process platelet components containing doses of 5.0 to 12.0 × 10^{11} platelets in a volume of 420 to 650 ml of 47 to 68% plasma and 32–53% PAS.
Aims: To evaluate the processing ranges for single donor or pooled apheresis PCs suspended in PAS and treated with the INTERCEPT TS platelet set.
Methods: Apheresis PCs (Amicus® and Trima®) were collected in 35–47% plasma and 53–65% PAS-3. One study was performed at the nominal dose (9.2–10.0 × 10^{11} platelets), volume (558–629 ml) in 65% PAS/35% plasma using single donor apheresis collections. Three studies were performed to evaluate the high dose and high volume condition (9.7–11.8 × 10^{11} platelets in 593–659 ml) using either single donor or pooled donations at nominal plasma content ratio or pooled donations at the high plasma content ratio. One study was performed to evaluate the low dose and low volume condition (5.5–5.9 × 10^{11} platelets in 432–449 ml) using pooled apheresis donations at nominal plasma ratio. Input PCs (n = 33) were treated with the INTERCEPT TS set by the end of Day 1 post collection; the incubation time in the Compound Adsorption Device (CAD) container ranged from 4 to 16 h and the INTERCEPT treated PCs were stored in 2 or 3 containers. Day 5 and 7 post-donation PCs were evaluated using a panel of *in vitro* platelet function assays and evaluated for pH and dose per the EDQM criteria (pH >6.4 and 75% of units having a dose ≥2 × 10^{11} platelets).
Results: INTERCEPT treated PCs met the EDQM platelet dose requirement in 100% of the 89 INTERCEPT units evaluated and 48 of 49 INTERCEPT units evaluated met the EDQM requirement for pH after 7 days of storage (not all INTERCEPT units were evaluated for pH). Platelet dose and volume recovery post-treatment ranged from 82% to 110% and 88% to 95%, respectively. Post-CAD amotosalen levels were within the requirement of ≤2 μM amotosalen (≤0.1–0.3 μM) across the CAD times evaluated (9.7–16.5 h). *In vitro* function data for apheresis PCs in PAS-3 treated in the INTERCEPT TS set were compared to data obtained from a multicenter trial evaluating single and double apheresis PCs in PAS-3 treated with the INTERCEPT DS set and demonstrated comparable *in vitro* function.
Summary/Conclusions: The INTERCEPT Blood System for Platelets using the TS set demonstrated acceptable dose and pH according to the EDQM criteria and maintained acceptable *in vitro* quality through 7 days of storage.
INTERCEPT Blood System for Platelets TS kit is currently not approved for use.

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OPERATIONAL VALIDATION OF THE PREPARATION OF PATHOGEN INACTIVATED DOUBLE DOSE BUFFY COAT PLATELET CONCENTRATES

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Background: Efficiency and cost effectiveness in the preparation of pathogen inactivated (PI) platelet concentrates (PC) require new and innovative production disposables. An "I-Platelet Pooling" (IPP) set (Kansuk) incorporating a Sepacell™ PLX 5 leukodepletion filter (Asahi Kasei) was developed to obtain a PC from up to 8 buffy coats (BC). This intermediate product can then be treated by a photochemical process utilizing amotosalen and UVA (INTERCEPT Blood System, Cerus) using Dual Storage (DS) processing sets to obtain 2 PI PC products for transfusion with less consumables and labor.
Aims: The objective of this study was to evaluate the use of the IPP set in combination with the DS set. The study was structured in two phases, "pilot" and "routine use" evaluation of double dose (DD) INTERCEPT treated platelets.
Methods: BC used for DD PC preparation were approximately 42 ml, 37% hematocrit and 0.8×10^{11} platelets. The pilot phase included the preparation of 15 DD BC PC in SSP+ (Macopharma) with the IPP set and Compomat G5 (Fresenius Kabi) with evaluation of platelet yield and recovery, plasma percentage, residual leukocytes and filtration time. The PCs were treated with INTERCEPT DS set and split in two doses. Platelet yield was measured. During routine evaluation, 50 DD BC platelets were prepared in the same way with measurements of platelet yields (pre & post INTERCEPT), residual leukocytes (n = 32) and time to process 3 DD products.
Results: During the pilot phase, DD PC had a volume of 410 ± 11 ml and plasma ratio of 40 ± 1%. The platelet yield was $5.2 \pm 0.4 \times 10^{11}$ with recovery of 72 ± 7% from the BC pool to the DD PC. WBC counts were all below 1×10^{6} with a separation and leukodepletion time of 4.5 ± 0.4 min. After INTERCEPT treatment and split, the volume of each PC was 192 ± 6 ml and the platelet yield above 2×10^{11} in all units ($2.5 \pm 0.2 \times 10^{11}$).
During routine phase, platelet volume was 399 ± 12 ml and platelet yield $5.4 \pm 0.5 \times 10^{11}$ in DD PC before and $2.5 \pm 0.2 \times 10^{11}$ after INTERCEPT. WBC counts in DD PC were $0.06 \pm 0.10 \times 10^{6}$, all below 1×10^{6}.
The time to produce 3 DD PC with 1 operator was 55 min.
Summary/Conclusions: All units produced during the evaluation phases using the I-Platelet Pooling set met the INTERCEPT guard bands (volume, platelet content, plasma ratio) for DD PC treatment using the DS set except for 3 of 65 that were slightly above (max. 9 ml) the required volume (300–420 ml). This can be addressed by minor adjustments in pool volume. The IPP set performed as expected without WBC outliers. PC obtained post INTERCEPT met EDQM guidelines. Processing 8 BC pools allows reducing the consumables used with acceptable time for obtaining a DD PC and the same INTERCEPT processing time as required for treating a single dose. By implementing this method, the process has been simplified and the processing time has been reduced.

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IN VITRO ASSESSMENT OF PLASMA-REDUCED SINGLE DONOR APHERESIS PLATELET CONCENTRATES: COMPARISON OF UVC-TREATED, Y-IRRADIATED AND UNTREATED PLATELET UNITS

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Background: Treatment of apheresis platelet concentrates (PCs) with UVC may enhance transfusion safety of platelets with respect to contamination with pathogens.
Aims: In vitro quality of UVC-irradiated PCs (UVC-PCs) was compared with that of y-irradiated PCs (y-PCs) and untreated PCs (AP-PCs) under routine production conditions.
Methods: 18 PCs were prepared from single donors with standard operation procedures (Amicus) using SSP+ (Macopharma, Mouvaux; France) as additive solution and divided into three groups (n = 6 each): UVC-PCs were treated with UVC within six hours after preparation using the THERAFLEX UV-Platelets system (Macopharma); y-PCs were y-irradiated with a minimum of 25 Gy; and AP-PCs were left untreated. Sampling for quality control parameters was done on day of preparation (day 0) and after six days of storage (day 6). The following parameters were examined on day 0: PC volume, platelet concentrations before and after UVC treatment, plasma content, residual erythrocyte and leucocyte counts, pH, swirling and sCD62 (with and without thrombin-receptor activating peptide [TRAP]/collagen activation); measurements on day 6 were: platelet concentration, pH, swirling, sCD62 and sterility testing.
Results: Mean volumes were 333 ± 5.4 ml in AP-PCs, 341 ± 7.1 ml in y-PCs and 329 ± 6.0 ml in UVC-PCs with platelet counts of 3.6 ± 0.5/unit, 3.8 ± 0.3/unit and 3.9 ± 0.3/unit, respectively. Residual plasma concentration ranged between 31% and 38%. Residual erythrocytes and leucocytes met the standard specifications for PC products in Germany. At the end of shelf life, the pH value of UVC-PCs was significantly lower (7.01 ± 0.05) compared to y-PCs (7.18 ± 0.04) and AP-PCs (7.17 ± 0.05). No differences were detected for sCD62 expression between the three PC types with and without TRAP/collagen activation. Tests for bacterial contamination were negative for all tested PCs.
Summary/Conclusions: Study results demonstrate that plasma-reduced UVC-treated apheresis PCs meet the standard specifications for PC products in Germany. The significantly lower pH value at end of storage may be attributable to a higher metabolic activity in UVC-PCs. The safety and efficacy of UVC-treated PCs is being evaluated in a clinical study.

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EVALUATION OF PATHOGEN REDUCED (AMOTOSALEN-UVA) POOLED CRYOPRECIPITATE AND CRYOPRECIPITATE-POOR PLASMA

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Background: A method was developed for the production of cryoprecipitate (CP) and cryoprecipitate-poor plasma (CPP) for efficient utilization of whole blood collections and increased availability of safer products for transfusion. A process was developed to produce pathogen reduced (PR) CP and CPP using amotosalen-UVA (INTERCEPT™ Blood System, Cerus Corporation, Concord, CA) using licensed INTERCEPT plasma. An in-vitro study was performed to evaluate the production of PR CP (3 unit CP) and two units of CPP using mini-pools of 3 units of ABO-matched previously PR frozen plasmas. Levels of Fibrinogen, coagulation factor FVIII and albumin content were measured to assess quality.
Aims: To evaluate the in vitro characteristics of pathogen-reduced cryoprecipitate and cryo-poor plasma.
Methods: Whole blood donations of 450 ml ± 10% (CPDA) were processed following local procedures. Plasma units were frozen within 8 h and stored at <−25°C. After thawing, 3 O positive plasma units were pooled into a standard 600 ml transfer container. The pooled plasma units were then treated with 150 μM amotosalen HCl and 3 J/cm^2 UVA, frozen for at least 1 h at <−25°C and thawed for 20–24 h in a 2–6°C refrigerator to precipitate cryo proteins. By routine centrifugation CPP was separated from CP, frozen and stored at <−25°C. In vitro tests were performed pre- and post-PR and post thaw.
Results: PR CP maintained levels of Fibrinogen (average 1,040 mg/3 unit mini-pool) and Factor VIII (average 175 IU/unit) which met the protocol-specified mean European therapeutic targets* >450 mg Fibrinogen and ≥150 IU factor VIII per unitary product. Fibrinogen levels of the PR CP 50% greater than the fibrinogen levels in current production 492 mg (current average for a pool of 3 single units transfused). PR CPP contained on average 288 ± 37 mg of Fibrinogen, 42 ± 14 IU of FVIII and 7.6 ± 0.6 g of albumin. PR CP is currently being used for acquired Fibrinogen deficiency replacement while as CPP is currently used for plasma exchange to treat patients with thrombotic thrombocytopenic purpura.
*For FVII content we applied the EU specifications for PR plasma and used the minimum activity of on average 50 IU/unit or 150 IU/pool.
Summary/Conclusions: PR jumbo CP can replace the currently used 3 single units. Pathogen reduction improves safety for patients in need of CP and CPP, it reduces risk associated with highly prevalent emerging pathogens and allows for making more CP as a source of Fibrinogen, and CPP available from whole blood donations at lower cost. A mini-pool of three CP units is equivalent to one industrial Fibrinogen concentrate, which contains 1 g of the protein. Additional applications for the use of PR CPP are feasible where albumin is indicated for transfusion.

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Abstract has been withdrawn.

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ASSESSMENT OF CELL QUALITY FOLLOWING RIBOFLAVIN AND ULTRAVIOLET LIGHT TREATMENT OF WHOLE BLOOD IN CITRATE-PHOSPHATE-DEXTROSE-ADENINE ANTICOAGULANT

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Background: The Mirasol® Pathogen Reduction Technology (PRT) System for Whole Blood (WB) is a CE marked device for extracorporeal treatment of WB. It uses riboflavin and ultraviolet (UV) light to reduce the infectious pathogen load and to inactivate white blood cells (WBC) in WB for transfusion. Recently, Mirasol treatment has been shown to significantly reduce the transmission of *Plasmodium spp.,* the causative agent for malaria, in the African Investigation of the Mirasol System (AIMS) randomized controlled clinical trial. *In vitro* testing of the system has also demonstrated significant reduction of other parasites and viruses, such as *Babesia spp*. and Human Immunodeficiency Virus (HIV). Hence, we believe that the system has the potential to improve the safety of whole blood transfusion. Currently, WB for treatment with the Mirasol system is collected in citrate-phosphate-dextrose (CPD) anticoagulant, but the anticoagulant citrate-phosphate-dextrose-adenine (CPDA-1) is more commonly used for WB transfusion. Given this preference, there is great interest in evaluating Mirasol treatment of WB in CPDA-1.
Aims: To demonstrate that adequate cell quality is maintained following Mirasol treatment of WB in CPDA-1.
Methods: After IRB approval was obtained, 14 units of WB were collected in CPDA-1 using standard collection sets at a local blood center. On the day of collection, 11 units were treated using the Mirasol PRT System and 3 units were left untreated as controls. Mirasol treatment involved transfer of the WB mixed with 35 ml of 500 μM riboflavin solution into an illumination bag and exposing it to an 80 J/mL_{RBC} UV energy dose with the Mirasol Illuminator. The units were sampled following collection, after treatment, and on every 7th day of refrigerated storage through 21 days for measurement of complete blood counts, blood gases and electrolytes. The % hemolysis was determined for evaluation against the Council of Europe (COE) criterion (<0.8%).
Results: After 21 days of storage, the mean hemolysis for Mirasol-treated units was 0.20 ± 0.11% (range 0.09–0.43), meeting the COE criterion. Among other parameters measured, the most notable difference was observed for potassium, where the concentration was substantially elevated in Mirasol-treated units (38.8 ± 4.1 mM) relative to untreated controls (20.4 ± 3.2 mM) ($P < 0.0001$).
Summary/Conclusions: Stored WB met the COE criterion of hemolysis < 0.8% at 21 days of storage, and potassium levels were similar to those seen in Mirasol-treated WB in CPD. This preliminary data suggests that Mirasol treatment of WB in CPDA-1 provides acceptable RBC cell quality through 21 days of storage. Further testing to characterize the hemostatic properties of the WB will be conducted to qualify an expanded indication for the CE marked system to allow treatment of WB in CPDA-1.

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ASSESSMENT OF PATHOGEN REDUCTION CAPACITY FOLLOWING RIBOFLAVIN AND ULTRAVIOLET LIGHT TREATMENT OF WHOLE BLOOD IN CITRATE-PHOSPHATE-DEXTROSE-ADENINE ANTICOAGULANT

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Background: The Mirasol® Pathogen Reduction Technology (PRT) System for Whole Blood (WB) is a CE marked device for extracorporeal treatment of WB. It uses riboflavin and ultraviolet (UV) light to reduce the infectious pathogen load and to inactivate white blood cells (WBC) in WB for transfusion. Recently, Mirasol treatment has been shown to significantly reduce the transmission of *Plasmodium spp.,* the causative agent for malaria, in the African Investigation of the Mirasol System (AIMS) randomized controlled clinical trial. *In vitro* testing of the system has also demonstrated significant reduction of other parasites and viruses, such as *Babesia spp*. and Human Immunodeficiency Virus (HIV). Hence, we believe that the system has the potential to improve the safety of whole blood transfusion. Currently, WB for treatment with the Mirasol system is collected in citrate-phosphate-dextrose (CPD) anticoagulant, but citrate-phosphate-dextrose-adenine (CPDA-1) is more commonly used for WB transfusion. Given this preference, we are evaluating the pathogen reduction performance of the Mirasol PRT System in treating WB in CPDA-1.
Aims: To demonstrate that pathogen reduction in CPDA-1 is equivalent or better compared to Mirasol-treated WB in CPD.
Methods: Pathogen reduction was assessed using the bacteriophage ΦX174 and the bacterium *Yersinia enterocolitica* (ATCC# 23715). ΦX174 is a model virus that has been selected to evaluate the consistency of the general mechanism for pathogen reduction under varying conditions. *Y. enterocolitica* is a human-relevant bacterium capable of growing at cold temperatures and therefore is relevant to WB, which is stored refrigerated at 4°C. For each pathogen, a minimum of N = 6 units in CPD and CPDA-1 were evaluated. In the case of *Y. enterocolitica,* autologous plasma was replaced with AB or type-matched complement-depleted plasma due to prior work demonstrating susceptibility to complement activity.
Units were inoculated with high titers of the appropriate pathogen prior to Mirasol treatment. WB was mixed with 35 ml of 500 μM riboflavin solution, placed into an illumination bag, and exposed to an 80 J/ml_{RBC} UV energy dose with the Mirasol Illuminator. Pre- and post-treatment samples were taken for enumeration of titers and calculation of log reduction. The mean log reduction values for WB in CPDA-1 were compared to values for WB in CPD using a Student's t-test.
Results: The mean ΦX174 log reduction values were 2.70 ± 0.28 (range 2.21–3.02, N = 7) and 2.70 ± 0.28 (range 2.46–3.22, N = 7) for CPDA-1 and CPD, respectively ($P = 0.9697$). For *Y. enterocolitica,* the mean log reduction in CPDA-1 was 2.66 ± 0.26 (range 1.92–3.10, N = 6) and in CPD was 2.86 ± 0.44 (range 2.36–3.56, N = 7) ($P = 0.3566$).
Summary/Conclusions: For the two pathogens tested, preliminary results show that mean log reduction values were equivalent to within the variability of the assay. The equivalence in pathogen reduction performance suggests that an expanded indication for the CE marked system can be pursued.

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PATHOGEN INACTIVATION OF WHOLE BLOOD (WB) DERIVED PLASMA WITH AMOTOSALEN/UVA: RESULTS FROM A VALIDATION IN VITRO STUDY

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Background: Pathogen inactivation (PI) of plasma increases the safety of plasma transfusion. To establish an alternative to quarantine plasma a PI technology suitable for blood banks can be used. Pooling of WB plasma before PI can increase the efficacy providing that the specifications of the EDQM's guide to the preparation, use and quality assurance of blood components for PI plasma (Factor VIII>50 U/dl; Fibrinogen-recovery >60%) and the requirements of the PI technology are met.
Aims: The aim of the validation study was to prove the feasibility and efficacy of producing PI plasma pooled from five WB derived fresh or thawed plasma units with or without leukocyte reduction (WBC-R) and processed with the INTERCEPT Blood System™ for plasma.
Methods: Four groups of PI plasma were produced by pooling five units each of ABO-identical WBC-R fresh plasma (group G 1) or previously thawed (G 2) or non-WBC-R fresh plasma (group G 3) or thawed (G 4). In each group at least one pool was of blood type O. Each Pool was split into two parts, each half with a maximum of 650 ml was separately pathogen-inactivated with amotosalen/UVA with the outcome of 3 units of PI-plasma of each part (six units per pool).
Results: Results are presented as mean±SD followed by mean values for G1, 2, 3 and 4. We produced 16 pools, four in each group, resulting in 32 PI processes and 96 end products with a volume of 200 ± 5 ml (197, 201, 200, 202 ml). WBC in the pool were below 1 × 10E6/L in filtered plasma (G1 andG2) and below 0.1 × 10 E9/l in the unfiltered plasma (G3 andG4). Residual amotosalen was below the threshold of 2 μmol/l in all products (0.8 ± 0.1). Retention of coagulation factors was good with values after INTERCEPT treatment and prior to storage of: F VIII 80 ± 16 IU/dl (83, 68, 79, 90), and fibrinogen 230 ± 21 mg/dl (235, 231, 223, 230). Fibrinogen

recovery on average was 90 ± 5% (90, 91, 89, 92) with values of 221 ± 20 mg/dl (222, 213, 231, 217) after treatment and 87 ± 6% (86, 84, 93, 86) after storage for six months. F VIII retention was also very good with 75 ± 22 IU/dl (83, 68, 79, 90) after storage. and fibrinogen-recovery. Other coagulation and inhibiting factors were also well preserved.

Summary/Conclusions: Each of the four groups of PI plasma met the specifications of EDQM and the requirements of the INTERCEPT Blood System™ for plasma. It is feasible, safe and efficacious to use a pool of five WB plasma units and resulting in six units after the INTERCEPT PI process with fresh and thawed plasma as well as WBC reduced and non-reduced plasma. This novel approach has also very positive economical implications.

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IN VITRO STORAGE QUALITY OF TRIPLE DOSE APHERESIS VS SINGLE DOSE WHOLE BLOOD DERIVED PHOTOCHEMICALLY TREATED PLATELET CONCENTRATES

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Background: Although the risk of viral transfusion-transmitted infections (TTI) from recognized pathogens is low today, bacterial contamination and emerging pathogens remain a threat to transfusion safety. The INTERCEPT™ Blood System (Cerus) was developed to prevent TTI by inactivating pathogens utilizing amotosalen in combination with UVA light. The triple storage (TS) set has been designed to treat double or triple dose platelet donations and is currently in development.

Aims: A study was conducted to evaluate the storage quality of triple dose apheresis platelet concentrates INTERCEPT treated with a TS set (ITAPC). The results will be compared with quality control data from pooled buffy coat platelet concentrates treated in single volume sets (SV) (IBCPC).

Methods: 6 triple dose apheresis platelet donations (9–11 × 10^{11} platelets) from normal volunteer donors collected with Trima Accel® platform (Terumo BCT) were suspended in 40% plasma/60% SSP+ (Macopharma), for INTERCEPT processing with TS sets to obtain after split 6 × 3 treated units. Six BCPC (2 to 4 × 10^{11} platelets) were processed by INTERCEPT in single volume (SV) sets. Platelet function on Day 1 and 5 was compared for the two products.

Results: Platelet concentrates from triple dose donations and pooled platelet concentrates treated with amotosalen had comparable platelet content (3.2 ± 0.2 × 10^{11} (1of 3 units) vs 3.0 ± 0.3 × 10^{11}). The volume of ITAPC was lower (195 ± 3 ml (1 of 3 units) vs 263 ± 5 ml). *In vitro* function was comparable. The pH (22°C) was 6.8 ± 0.14 (D1) to 6.9 ± 0.07 (D5) in ITAPC slightly decreased compared to IBCPC [7.0 ± 0.03 (D1) to 7.1 ± 0.06 (D5)].

ITAPC showed an increased expression of activation marker CD62 after stimulation with ADP (40 μM) ([D1] 24.0 ± 5.3% without stimulation, 55.5 ± 11.4% stimulated, [D5] 34.5 ± 9.5% without to 51.4 ± 6.6% after stimulation). In IBCPC a mean expression of CD 62 after same stimulation increased by 43.8 ± 9.7% (D1) to 53.9 ± 5.1% (D5). Both platelet products maintained their ability to be activated by ADP.

Concentration of glucose decreased from 104.7 ± 33.9 mg/dl (D1) to 32.4 ± 27.0 mg/dl (D5) in ITAPC and from 119.5 ± 7.4 mg/dl (D1) to 82.3 ± 9.0 mg/dl (D5) in IBCPC. In comparison concentration of lactate increased in ITAPC up to 116.9 ± 15.6 mg/dl (D5), in IBCPC up to 160.0 ± 16.1 mg/dl (D5). All tested units were negative in bacterial cultures in both groups.

Summary/Conclusions: Pathogen inactivated apheresis platelets processed using the TS set and pooled whole-blood derived platelet concentrates processed in SV set retained adequate *in vitro* function for up to 5 days and met the criteria of the "Council of Europe Recommendation N°.R (95) 15" as well as the German guidelines. The possibility to treat high dose apheresis platelets with as single processing set is operationally convenient.

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PATHOGEN INACTIVATION SYSTEMS IN POLISH BLOOD TRANSFUSION CENTERS IN 2012–2015

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Background: An integral part of the Health Program of the Republic of Poland announced by the Polish Minister of Health in 2009 was to assure self-sufficiency in blood, blood components and blood products and to supply Polish blood establishments with safe blood components subjected to pathogen inactivation. Some Polish blood transfusion centers had launched the implementation of pathogen inactivation using the Theraflex MB Plasma system already in 2008. In 2009 several blood transfusion centers installed pathogen inactivation systems dedicated first to plasma inactivation and then to inactivation of platelet concentrates (PC). The ultimate aim of the implementation of pathogen inactivation methods was to obtain safe plasma that could serve as alternative for quarantine plasma. The currently observed tendency is the unchanging volume of quarantine plasma related to the increase in the number of first time donors. Currently in Poland there are 12 Theraflex MB Plasma systems and 28 Mirasol PRT systems installed in 11 of the 23 blood transfusion centers.

Aims: Evaluation of the status of implementation of pathogen inactivation methods in the Polish blood transfusion centers in 2012–2015 and the usage of inactivated blood components in clinical practice.

Methods: A template-questionnaire was developed and distributed to all blood transfusion centers. The completed questionnaires provided data on types of inactivation methods, validation results for plasma and PC as well as statistical data referring to: the number of annually collected plasma units (whole blood and automatic), number of plasma units subjected to inactivation, number of PC units (pooled or automatic), number of PC units subjected to inactivation.

Results: During the years 2012–2015 the percentage of inactivated plasma units decreased from: 50% and 60% in 2012 (2 Warsaw centers), 30% and 50% in 2013 (in the same 2 Warsaw centers respectively), 25% in 2014 (only 1 of the 2 Warsaw centers) to about 10% in 2015 in the same 2 Warsaw centers. In the remaining 21 blood transfusion centers the implemented inactivation systems were in less than 1%. The following amounts of inactivated blood components were issued for clinical use nationwide: 4.73% – in 2012; 7.22% – in 2013, 8.74% – in 2014, 9.47% – in 2015 of inactivated units of fresh frozen plasma (FFP) and for of inactivated PCs: 12.58% – in 2012, 13.29% – in 2013, 12.06% – in 2014, 11.47% – in 2015.

Summary/Conclusions: Although a slight increase was observed in the number of inactivated plasma units issued for clinical use in 2012 – 2015, the pathogen inactivation systems that were implemented in the Polish blood transfusion centers are not exploited to the full. Training for physicians seems therefore necessary to enhance the awareness of the opportunity of transfusing patients with much safer blood components.

P-269

Abstract has been withdrawn.

P-270

IMPLEMENTATION OF DOUBLE DOSE PATHOGEN INACTIVATED PLATELETS IN ROUTINE WITH PRODUCTIVITY AND COST OPTIMIZATION

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Background: The INTERCEPT Blood System (IBS, Cerus) utilizes a photochemical treatment with amotosalen and UVA to inactivate contaminating viruses, bacteria, parasites and leucocytes in platelet concentrates and plasma. The Dual Storage (DS) set allows the treatment of a double dose (DD) of platelets.

Aims: The Blood Center of Aalborg between October 2012 and October 2014 (period 1) prepared platelet concentrates (PC) from pools of 4 buffy coats (BC) with Tacsi automated platform (Terumo BCT). After a decision to implement INTERCEPT, the production method was adapted to prepare DD PC from pools of 7 BC, first with Tacsi (November 2014 to January 2016 – period 2) then with a manual pooling set (Macopharma) (February to September 2016 – period 3). We compared QC data, productivity and cost for the 3 periods.

Methods: BC prepared on Macopress separators (Macopharma) had the following average specifications during period 1: volume 62 ml, Hct 50%, plt count 1×10^{11}. For periods 2 and 3, this was adapted to: volume 45 ml, Hct 35%, plt count 1.1×10^{11}. Single dose (SD) PC were produced on Tacsi from pools of 4 BC with 300 ml SSP+ (Macopharma) during period 1. DD PC produced in period 2 from 7 BC on Tacsi or in period 3 with a manual pooling set and 280 ml SSP+ were photochemically treated with INTERCEPT DS sets to yield two pathogen inactivated PC.
Results: Production during period 1 (4 BC, Tacsi) was 250 ± 20 SD PC with 54 ± 22 irradiated PC per month. During period 2 (7 BC, Tacsi, IBS), it was reduced to 142 ± 13 DD PC (equivalent to 284 ± 26 SD PC) without need for irradiated products. It remained stable during period 3 (7 BC, manual separation, IBS) with 144 ± 16 DD PC. Platelet content per PC was $293 \pm 43 \times 10^9$ (n = 258), $266 \pm 40 \times 10^9$ (n = 158) and $272 \pm 39 \times 10^9$ (n = 92) for the 3 periods so the impact of shifting from SD to DD and treating with IBS was a reduction of about 9% of the platelet content per PC regardless of the platelet preparation method used. Residual leucocytes met the requirement of $<1 \times 10^6$/unit in 90% of the PC during the 3 periods. The cost of all disposables used per platelet dose when implementing INTERCEPT increased by 25% in period 2 but decreased by 7% in period 3 both compared with period 1
Summary/Conclusions: The production of DD BC Platelets with INTERCEPT was successfully implemented. BC characteristics were adapted (reduction of volume and haematocrit) and platelet production process optimized to obtain two doses of platelets from 7 BC instead of one dose from 4 BC with only 9% reduction in yield. The monthly production of PC became more efficient when implementing the DD method with IBS (43%). Gamma irradiation of PC was stopped. The adoption of the DD BC production method with a manual pooling process is less expensive despite the addition of a pathogen inactivation process.

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RIVOFLAVIN AND UV LIGHT TREATED PLATELETS ENSURE AN ADEQUATE TRANSFUSION DOSE, SIMILAR TO UNTREATED PLATELETS

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Background: Pathogen Reduction Technology (PRT) based on riboflavin and UV light for platelet (PLT) and plasma components enhances blood safety by inactivating viruses, bacteria, parasites as well as donor leukocytes. Additionally, PRT for platelet (PLT) components has remarkable advantages such as replacing bacterial contamination screening tests, CMV tests, gamma irradiation and also reducing PLT transfusion reactions. However, it has been reported that the dose of PRT-treated PLTs is lower that non-PRT treated PLTs and that decline of transfused dose could theoretically have an impact on the effectiveness of the transfusion.
Aims: In order to investigate whether the transfusion PLT dose of PLTs treated with riboflavin and UV light was inferior or not to the transfusion PLT dose of untreated PLTs, a retrospective analysis was conducted into PLT dose giving to patients of the Balearic Islands University Hospital during the 3 years before and after the implementation of PRT based on riboflavin and UV light for platelet
Methods: Since 2013, our regional Blood Bank provides PLT components prepared with riboflavin and UV-light (Mirasol PRT system, Terumo BCT, Lakewood, CO, USA) for transfusing patients with thrombocytopenia. We studied the PLT dose per unit, the number of PLT concentrates and the total dose of PLTs received per patient at the Balearic Islands University Hospital during 2013–2015 and compared with the data of a three-year control period before riboflavin and UV-light implementation (2005–2007).
Results: A total of 14,407 platelet transfusions (6,357 during the pre-PRT period and 8,050 during the post-PRT period) administered to 2,977 patients (1,479 patients transfused during the pre-PRT period and 1,498 during post-PRT period) were included in the retrospective study. The mean dose of PLTs per unit was very similar for both periods (pre-PRT 3.26 vs post-PRT 3.19; P = 0.11). The mean number of PLT concentrates per patient (pre-PRT 4.34 vs post-PRT 5.79; P = 0.32) and the total dose of PLTs received by the patient (pre-PRT 14.15 vs post-PRT 18.46; P = 0.17) were also not statistically significant for each period. The increased number of PLT concentrates transfused during the PRT period (post-PRT 8,050) may be attributed to the slightly increment in the number of patients receiving PLTs (pre-PRT 1,479 vs post-PRT 1,498; P = 0.95) and may also be due to the start of the allogeneic bone marrow transplant program during the PRT period at the Balearic Islands University Hospital. However, the increment observed was not statistically significant (pre-PRT 6,357 vs post-PRT 8,050; P = 0.06).
Summary/Conclusions: The mean dose of PLTs per unit, the mean number of PLT concentrates per patient and the total dose of PLTs received by the patient was similar during both periods, pre-PRT and post-PRT periods. Therefore, we can conclude that, in our experience, the riboflavin and UV light system for PLT does neither decrease significantly the PLT transfusion dose nor increase the number of PLT concentrates received per patient.

P-272

Abstract has been withdrawn.

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EFFECTIVENESS OF AMOTOSALEN/UVA LIGHT PATHOGEN-REDUCED PLATELETS TRANSFUSED TO ADULT PATIENTS

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Background: Platelets transfusions are an important tool for the prevention and treatment of bleeding in thrombocytopenia and / or thrombocytopathy. In contrast to decreasing red blood cell and plasma transfusions in developed countries, the incidence of platelet transfusions increases steadily. To mitigate the risk of transfusion-transmitted infections and Graft-vs-Host Disease, Amotosalen/UVA light pathogen inactivation (PI) technology (INTERCEPT Blood System, Cerus Corporation, Concord, USA) has been implemented in many blood banks in the Republic of Bashkortostan (RB) as an additional layer of safety.
Aims: To study the effectiveness of pathogen-inactivated platelets transfusions in the adult hospitals of the RB region.
Methods: The answers for the questionnaire "Platelet transfusions survey" were collected from 16 hospitals in the RB region covering PI transfusion events between the 14.01.2016 and the 30.09.2016. 246 patients received 1,519 Amotosalen/UVA light-treated platelet units. Main diagnoses: neoplasms – 144 patients (58.5%), diseases of the blood and blood-forming organs – 36 patients (14.6%), certain conditions originating in the perinatal period – 26 patients (10.6%). For the present study 563 transfusions have been excluded:
- 522 – to patients with body surface area less than 1 m^2;: - 41 – with underfill transfusion protocol.
The results were processed using descriptive statistics with a significance level of 0.05.
Results: 70 (7.3%) units have been transfused to stop bleeding, in other cases 886 (92.7%) units were transfused for prevention of bleeding.
In therapeutic and prophylactic transfusion recipients was no significant difference in body surface area and the average number of previous transfusions. Only the number of first platelet transfusions to control bleeding was 13.3% higher than for bleeding prevention (OR 2.14, 95% CI – 1.24–3.67, P < 0.01).
The platelet count before treatment transfusions was at 21.6% lower in the therapeutic transfusion group compared to the group of preventative transfusions. A similar ratio is maintained for the concentration of platelets after transfusion.
Corrected count increment (CCI) after 24 h in the investigated groups did not differ.
The preventive transfusion group the CCI directly correlated to the concentration of platelets both before (r = 0.157; P < 0.001), and after (r = 0.729; P < 0.001) transfusions.
In therapeutic transfusions group the CCI does not correlate with the concentration of platelets prior to transfusion and directly correlates with platelet concentration post (r = 0.748; P < 0.001) transfusion.
Summary/Conclusions: Therapeutic transfusions were characterized by increased part of first procedures and decreased platelet counts before transfusion as well as the lack of corrected count increment (CCI) correlation with the initial concentration of platelets.
The preventive transfusion group CCI directly correlated to the concentration of platelets pre- and post transfusion. Direct correlation between CCI and the initial platelet concentration indicates a lack of consumption of transfused platelets and the possible redundancy of prophylactic transfusions. No adverse transfusion reactions have been registered after 956 pathogen-reduced platelets transfusions.

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BACTERIAL KILL CAPABILITIES OF TWO PATHOGEN INACTIVATION SYSTEMS

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Background: Bacterial culture screening using the BacT/ALERT system of all NHS Blood and Transplant (NHSBT) platelet components has contributed to a marked reduction in bacterial transfusion transmissions, with only one confirmed transmission from testing over 1.2 million units (McDonald, *et al*., Transfusion 2017). Pathogen inactivation (PI) offers a potential alternative to bacterial screening. The NHSBT National Bacteriology Laboratory (NBL) has evaluated the Cerus Intercept and Terumo BCT Mirasol pathogen reduction systems to establish the maximum contamination level that can be effectively treated.

Aims: The aim was to determine the maximum bacterial concentration in platelet components that could be completely inactivated by each treatment system, with sterility maintained until the end of shelf life.

Methods: Bacterial isolates from transmission and routine platelet component screening were used for the evaluation, with ten and twelve species assessed for treatment using Mirasol and Intercept, respectively. The bacterial species were seeded individually into buffy coat-derived pooled platelets (65% additive solution, 35% plasma) in the concentration range of 10^{-1}–10^{7} CFU/ml. In order to provide multiple homogeneous units per species, units were pooled and divided into equal volumes. Three spiked units were prepared per concentration, with two treated as per the manufacturer's instructions, and the third an untreated control. Bacterial count was determined post-seeding, immediately prior to and following treatment and at the end of the seven day storage shelf life. The inactivation capability was determined as the seeded concentration in which no bacterial growth was observed at the end of shelf life in both replicates.

Results: For Intercept, the inactivation capabilities for each species, expressed in CFU/ml, were as follows: *Bacillus cereus* $<10^{3}$, *Pseudomonas aeruginosa* 10^{3}, *Serratia marcescens* 10^{3}, *Klebsiella pneumoniae* 10^{5}, *Escherichia coli* $>10^{6}$, *Listeria monocytogenes* $>10^{7}$, *Staphylococcus aureus* $>10^{7}$, *Staphylococcus epidermidis* $>10^{7}$, *Streptococcus bovis* $>10^{7}$, *Streptococcus dysgalactiae* $>10^{7}$, *Streptococcus mitis* $>10^{7}$, *Streptococcus pneumoniae* $>10^{7}$.

For Mirasol, the inactivation capabilities were: *Escherichia coli* $<10^{1}$, *Klebsiella pneumoniae* $<10^{1}$, *Serratia marcescens* $<10^{1}$, *Staphylococcus aureus* $<10^{1}$, *Streptococcus dysgalactiae* $<10^{1}$, *Listeria monocytogenes* 10^{1}, *Streptococcus bovis* 10^{1}, *Streptococcus mitis* 10^{1}, *Staphylococcus epidermidis* 10^{2}, *Streptococcus pneumoniae* 10^{4}.

It was observed that, on numerous occasions with both systems, the presence of bacteria was not detected immediately after treatment, but bacterial numbers were elevated at the end of shelf life.

Summary/Conclusions: Our data indicate that PI systems have a limit to their inactivation capabilities and this is species dependent. Intercept was shown to have a higher inactivation capability than Mirasol for the species studied. As such, Mirasol will need to be performed within a shorter time frame post-blood collection to ensure effective inactivation. For both systems, PI will need to be performed as soon as is operationally feasible to reduce the likelihood of bacterial growth occurring beyond the inactivation threshold of the system. We suggest that terminal sterility needs to be the 'gold standard' for bacterial assessment of PI systems as growth may be undetectable immediately following treatment. Further investigations are planned linked with growth kinetic studies to determine the most appropriate window period at which to treat donations for each system.

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EFFECTIVE PATHOGEN INACTIVATION IN TRIPLE SET KITS FOR PLATELETS SUSPENDED IN PLATELET ADDITIVE SOLUTION (PASIII)

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Background: A photochemical treatment process utilizing amotosalen and low energy ultraviolet A (UVA) light, was developed to inactivate pathogens and residual leukocytes in platelet components (PC). The INTERCEPT™ Blood System for Platelets, received CE mark for PC in 2002, and was approved in the US in 2014. Approximately 1.7 million kits have been sold since their approval for the preparation of inactivated components and comprise Small Volume (SV; 255–325 ml), Double Dose (DD; 300–390 ml) and Large Volume (LV; 375–420 ml) kits designed to cover platelet doses between 2.9 and 8.0 × 10^{11}. A new Triple Storage (TS) kit that was designed to expand the dose range to 12.0 × 10^{11} platelets and the maximum volume to 650 ml, generating either 2 or 3 doses of pathogen inactivated PC, was validated for efficacy of Pathogen Inactivation (PI) and function.

Aims: The objective of this study was to determine the effectiveness of PI at the worst case scenarios of high plasma content, high volume, and high platelet dose in the TS and the comparison with the results obtained for smaller platelet doses under nominal conditions.

Methods: For each experiment, a platelet pool with 10–12 × 10^{11} platelets was prepared at a final volume of ~650 ml in 47% plasma/53% PASIII. These conditions result in a final concentration of amotosalen at the lower limit of the range (135 μM *vs*. nominal of 150 μM). The platelet units were inoculated with high titers of a virus or a bacterium and processed. The results were compared to control samples taken prior to the addition of amotosalen and illumination of the PC. Control and test samples were serially diluted and inoculated at the appropriate conditions to determine pre- and post-UVA pathogenic titers. Log_{10} reduction was calculated as the difference between the mean titers in pre-UVA samples and post-UVA samples. The results (n = 4) were compared with the values obtained for previous inactivation studies under nominal conditions.

Results: The inactivation potential for representative viruses (bovine viral diarrhea, bluetongue and adenovirus-5) and bacteria (*E. coli, K. pneumonia and S. aureus)* were evaluated. The Log_{10} reductions obtained were: for the viruses: >5.6, >5.9 and >5.8, and for the bacteria >7.0, 6.7 and >7.7, respectively. Those results compare well with the CE mark inactivation commercial claims, of the same pathogens under nominal conditions of >6.0, >5.0, >5.9 for the viruses and >6.4, >5.6 and 6.6 for the bacteria in the same medium, indicating that performance of inactivation in volumes and platelet count necessary for the TS is not affected to any significant extent, even in the extreme limits of the ranges.

Summary/Conclusions: Inactivation of pathogens in platelet concentrates treated with the INTERCEPT TS kit is similar to those achieved under nominal conditions, even at extreme ranges of volume, plasma and platelet dose.

Novel blood products

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THE RELATIONSHIP BETWEEN DESIALYLATION OF PLATELETS AND TPO PRODUCTION IN HEPATOCYTES WITH STORAGE TEMPERATURE AND DURATION

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Background: The clearance of platelets in the body is usually occurred at the reticuloendothelial system. Recently, however, Hoffmeister et al reported that the circulatory lifespan of the platelets is determined by sialic aid loss that triggers platelet removal by hepatic Ashwell-Morell receptor (AMR).

Aims: In this study, we measured the desialylation of platelets by incubating platelets for 5 days at room temperature and at 4C, then comparing them with the degree of TPO uptake in AMR while coculturing the stored platelets with HepG2 cells. And we investigate whether the storage of platelets at room temperature is superior to that of refrigerated storage

Methods: Five healthy blood donors were donated whole blood, and platelet concentrates were prepared. The platelet concentrates were divided into two groups. One group was refrigerated and one group was kept at room temperature for 5 days. *Ricinus communis agglutinin I* (RCA-I) was measured using a flow cytometer for desialylation of platelets. TPO mRNA expression was measured in the hepatocyte by real-time PCR after 6 h of reaction with the platelets cocultured on HepG2 cells. TPO concentrates were measured by Magnetic Luminex® performance assay in the culture supernatant.

Results: Refrigerated platelet showed more desilylation than those stored at room temperature. And, by the storage duration of 5 days (D + 5), lectin binding ratio was increased proportionally by time course. The HepG2 cells reacted with aged or refrigerated platelets produced more TPO mRNA than those reacted with fresh (D + 0) platelets. However, The TPO concentration in the supernatant increased with storage, but there was no statistically significant difference between the two groups.

Summary/Conclusions: Our study demonstrated that desialylation of platelets occurred more frequently in aging and refrigerated storage. And we confirmed the increase in intrahepatic TPO mRNA due to the clearance of stored platelets. These experiments were the first attempts in *in vitro* models using human platelet.

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PRODUCTION AND QUALITY OF ALLOGENEIC PLATELET GEL PRODUCED BY PRECIPITATION OF EXPIRED APHERESIS PLATELET CONCENTRATES

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Background: Allogeneic platelet gel can be used to treat topical wounds and skin infections. Platelet gel is produced from platelet concentrates through addition of a clotting agent. Platelet gel contains growth factors derived from platelets, which are released onto the wound bed thereby facilitating healing. When preparing platelet gel, a cold precipitation method can be employed to increase the fibrinogen concentration, which may lead to platelet gel with better quality.
Aims: The aim of this study was to investigate the feasibility of using a precipitate from platelet concentrates to produce platelet gel.
Methods: Double apheresis platelets at day-6 post-collection were used to produce matching pairs (n = 9). Both units were frozen at −80°C. One unit was thawed at 37 °C and used as a baseline. The other unit was thawed at 2–6°C to produce a precipitate, which was separated from the supernatant by centrifugation. Samples were obtained from the baseline and the precipitate for testing. The activities of factor II (FII), FVIII, FXIII, protein C and the concentration of fibrinogen were measured using a coagulation analyser. Platelet derived growth factor (PDGF)-BB, transforming growth factor (TGF-β1), and basic fibroblast growth factor (bFGF) were measured using ELISAs. Platelet gel was produced by addition of 1% calcium gluconate. The viscoelasticity of the resulting platelet gel was measured by thromboelastography for 60 min. Data indicate mean and standard deviation. Data were analysed using two-tailed Wilcoxon t-tests and two-tailed Spearman correlations.
Results: The average count of apheresis platelets prior to freezing was $1.496 \pm 147 \times 10^9$ platelets/l. The volume of the baseline was 172 ± 12 ml whereas the volume of precipitate was significantly reduced to 32 ± 0 ml (P = 0.0039). The precipitation process resulted in a significant decrease in the activity of FII (baseline, 85 ± 10%; precipitate, 79 ± 8%; P = 0.0039), FXIII (baseline, 61 ± 25%; precipitate, 36 ± 10%; P = 0.0156) and protein C (baseline, 90 ± 19%; precipitate, 81 ± 17%; P = 0.0039). The activity of FVIII was not significantly altered (baseline, 43 ± 12%; precipitate, 54 ± 23%; P = 0.0898). The precipitation process resulted in a significant increase in the concentration of fibrinogen (baseline, 3 ± 1 g/l; precipitate, 5 ± 2 g/l. P = 0.0078). The concentrations of PDGF-BB (P = 0.0039), TGF-β1 (P = 0.0078), and bFGF (P = 0.0039) were also significantly increased. Addition of calcium gluconate initiated clot formation (R time) after 13 ± 6 or 10 ± 4 min in either baseline or precipitate respectively (P = 0.2031). The clotting speed (alpha angle; baseline, 27 ± 9 deg; precipitate, 57 ± 15 deg; P = 0.0195) and clot strength (MA; baseline, 40 ± 10 mm; precipitate, 58 ± 13; P = 0.0039) of the platelet gel derived from the precipitate were significantly increased compared to gel derived from the baseline. Fibrinolysis was not detected in any samples. The concentration of fibrinogen correlated significantly with clotting speed (P = 0.0060, r = 0.6205) and clot strength (P = 0.0227, r = 0.5331).
Summary/Conclusions: The use of precipitate from expired apheresis platelets to prepare platelet gel is promising. The usage of platelet concentrates manufactured under good manufacturing practice environment can result in allogeneic platelet gel with consistent quality. This in turn may enable the conduct of clinical trials to evaluate the efficacy of platelet gel for wound healing.

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AUTOLOGOUS SERUM EYE DROPS: FIVE YEARS EXPERIENCE OF MANUFACTURING AND APPLICATION

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Background: In severe cases of dry eyes, such as in GvHD, autologous serum eye drops are the "ultima ratio" and in many patients highly efficient in treating and preventing symptoms such as pain and visual disturbances.
Since 2012 our centre manufactures autologous serum eye drops in a closed system with a manufacturing license of the local authority.
Aims: We retrospectively evaluated our experiences in this 5 year period.
Methods: The number of patients, the respective disease / indication, the amount of donations per patient, side effects of donation, problems in logistics, clinical benefit and side effects, were evaluated.
Results: Between 2012 and 2016 in total 284 patients referred by the treating ophthalmologist were accepted for autologous blood donation; 602 products were manufactured. In the year 2016 83 patients donated (3 pt donated 4×, 20 pt 3×, 37 2× and 23 one time). The donations were processed in a closed system (Meise, Schalksmühle, Germany) as reported from our group before. The division in "one day dose" containers was refined so that up to 130 "ophthiols could be prepared from one donation of 500 ml whole blood. Quality control was performed on each donation: no bacterial contamination was detected.
Loss of product occurred in very few cases: initially due to leakage of the plastic bags during centrifugation. Some products were lost because of logistic problems: due to German pharmaceutical law products have to be delivered via a local pharmacy, chosen by the patient. Some of the patients came from distant parts of Germany. Very few products were lost due to thawing (breaking of the cold chain due to unforeseeably prolonged shipment on dry ice or dysfunction of freezers in private pharmacies).
Most of the patients reported a substantial relief of the symptoms. No serious side effects were observed or reported neither for donation nor for application. In 2 patients a decreased therapeutic effect was discussed in one subsequent donation.
Summary/Conclusions: Conclusion: In long term evaluation autologous serum eye drops are a save therapeutic option for severe cases of dry eye, which cannot be treated by other therapies.

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CHANGES IN GROWTH FACTOR CONTENT OF HUMAN SERUM FOR USE AS EYE DROPS DURING FROZEN STORAGE FOR 1 YEAR

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Background: Growth factors are thought to be among the active components in serum used for treatment of dry-eye syndrome. Stability of growth factors during frozen storage in mini containers (140 µl) is unknown. If these products can be stored at −18°C it will be feasible to store this product in 3-star household freezers, making the product available for patients in need of serum eye drops.
Aims: The purpose of this study is to demonstrate stability of growth factor content in human serum during longtime storage at −18°C or <−25 to −35°C packed in a new micro dose device for single use as eye drops.
Methods: Serum produced from 500 ml whole blood donations from non-remunerated healthy donors was quickly frozen. After frozen storage at <−25°C for 3–12 months and controlled thawing, six different sera were used to fill a large number of mini (140 µl) containers, which were refrozen and stored at either −18°C or <−25°C. During storage at 3 months intervals, samples were tested for several growth factors, using Magpix® Luminex Multiplex assays and compared to control samples stored at <−80°C. Growth factors tested were PDGF-AA&AB/BB, TGF-ß1/2/3, VEGF, EGF, FGF2. The study was a fact-finding study, without preset acceptance criteria.
Results: PDGF-AB/BB and TGF-ß1 were the most abundant growth factors, on average 35, resp. 40 ng/ml. Also PDGF-AA was detected at relatively high concentration in human serum, on average 11 ng/ml. TGF-ß2, EGF and VEGF were detected at relatively low values, resp. 3 ng/ml, 0.5 ng/ml and 0.3 ng/ml. Average levels of FGF2 and TGF-ß3 were close to detection limit (<0.2 ng/ml). The controls stored at <−80°C showed for all growth factors close to 100% of the initial values in samples at T = 0 (moment of filling mini containers). For serum stored at <−25°C for up to 12 months, most factors showed less than 2% decrease, except for PDGF-AA and TGF-ß2, showing 6% resp. 3% lower values. For serum stored at −18°C the values for TGF-ß1, EGF and VEGF were stable, whereas PDGF-AB/BB, PDGF-AA and TGF-ß2 showed a decrease of resp. 9, 17 and 3%.

Summary/Conclusions: Human serum eye drops can be stored in the new micro dose device at −18°C (3-star household freezers) or <−25°C (professional freezers) for at least one year after preparation without large decreases in growth factor content. The maximum decrease was found for PDGF-AA in serum stored at −18°C. It is yet unknown if the tested components add to the *in vivo* effectiveness of serum eye drops and what the minimal concentration is to ensure *in vivo* effectiveness. Further stability testing in combination with *in vitro* and *in vivo* application is required to extend the shelf-life beyond 1 year.

Transfusion transmitted infections

Screening strategies for TTI

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THE ROLE OF THE BLOOD DONATION ARCHIVE REPOSITORY AT THE IRISH BLOOD TRANSFUSION SERVICE (IBTS)

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Background: The Irish Blood Transfusion Service (IBTS) introduced the retention of a donation archive repository sample in 1993. Currently the archive sample is retained and stored for an indefinite period. The archive repository is mainly used in the completion of Nucleic Acid Testing (NAT) and/or Virology investigations (including yield cases) either as look-back testing on the previous negative donation or for additional confirmation testing on the index donation. The other main use of the archive repository is its role in traceback investigations of potential transfusion transmitted infections (TTI). The indefinite storage of all archive samples is currently under review at the IBTS.
Aims: The aim of the study is to evaluate the role of the donation archive repository at the IBTS in both lookback and traceback investigations.
Methods: Records of all donor lookback and traceback investigations were examined for a 10-year period (2007 through 2016) to determine the role of the archive repository in the investigation. The age of the archive retrieved was also determined.
Results: Over a ten year period (2007–2016), a total of fifty one archive retrievals were requested as part of look-back assessments at the IBTS. The microbiological markers investigated included Hepatitis B Virus (HBV-53%), Human Immunodeficiency Virus 1 and 2 (HIV-1/2–12%), Hepatitis C Virus (HCV-6%), Syphilis (SYPH-12%), Hepatitis E (HEV-13%), Toxoplasma gondii (Toxo-2%) and Varicella Zoster Virus (VZV-2%). Thirty seven of these lookback assessments involved donors who had seroconverted since their last donation. All thirty seven assessments excluded the previous donation as a risk to any recipient. The seroconversion time period between donations ranged from <1 month to 11 years. The archive repository also helped in the serological investigation of two donors who developed Toxoplasmosis and Varicella Zoster infections (Shingles) soon after donation.
Ten traceback investigations into potential TTIs required further testing on forty six archive donations. The microbiological markers investigated included HIV-1/2, HBV, HCV, HEV and Toxoplasmosis. The use of the archive repository in suspected TTI investigations facilitated the exclusion of 97% of the investigated archived samples as the source of a potential TTI. One archive sample is still under investigation. From the study it was determined that 94% of the archive samples retested in lookback investigations were stored for ten years or less. In relation to traceback investigations, 76% of the archive samples retested were stored for ten years or less.
Summary/Conclusions: It can be concluded that the IBTS archive repository plays a very important function in both lookback and traceback investigations. Based on this review, a ten year archive storage policy would allow 94% of lookback investigations which required testing on a donation archive repository sample to be completed. This would be in keeping with the guidance requirement for each Blood Service to maintain an archive of sufficient duration to enable approximately 75% of lookback requests to be investigated, recommended by the UK Standing Advisory Committee on Transfusion Transmitted Infection (SACTTI).

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SCREENING OF HEPATITIS E VIRUS RNA WITH A TRANSCRIPTION-MEDIATED AMPLIFICATION ASSAY IN BLOOD DONORS IN HOKKAIDO, JAPAN

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Background: We reported two cases of transfusion-transmitted hepatitis E in Hokkaido where hepatitis E is most prevalent in Japan. Thereafter, hepatitis E virus (HEV) RNA screening (HEV NAT) for research by 20-pool-NAT (20P-NAT) has been conducted in blood donors in Hokkaido, Japan since 2005. In August 2014, an individual-NAT (ID-NAT) system was implemented replacing 20P-NAT.
Aims: To clarify the current status of HEV infection among blood donors in Hokkaido, Japan.
Methods: From August 2014 to December 2016, all individual blood donors in Hokkaido were screened for HEV using the Procleix HEV assay which is based on a transcription-mediated amplification (TMA) assay and performed on the Panther system, a fully automated platform. Blood samples that tested positive for HEV RNA were also tested for the presence of IgM and IgG anti-HEV by a commercial ELISA kit, and HEV RNA load by in-house real-time RT-PCR. A partial sequence of 412 nucleotides of HEV ORF2 region was determined for HEV strains from the HEV-positive donors by direct sequencing. Questionnaires were sent to the HEV RNA-positive donors to collect data on their history of intake of animal meat before the index donation.
Results: Up to December 2016, more than 631,000 donors had been screened using HEV TMA. Of these, 442 (0.07%) donors were initially reactive. Retesting of these 442 samples by the same method showed that 234 and 208 samples were reactive and non-reactive, respectively. Four non-repeat reactive samples were reactive by in-house real-time RT-PCR. Based on this analysis, it was determined that 238 (0.038%) donors were true-positives for HEV RNA. In ID-NAT samples (n = 238), mean viral load was 2.4 ± 1.4 log IU/mL (range, <2.0–6.0 log IU/ml) and, due to the increased sensitivity, HEV viral load was less than 2.0 log IU/ml in half of the HEV-positive donors. Of 238 donors, 164 had neither IgM nor IgG anti-HEV and they were presumably in the early stage of HEV infection at the time of donation. Phylogenetic analysis of HEV sequences isolated from blood donors in Hokkaido showed that 86% were classified into genotype 3 (subgenotypes 3a, 3b and 3e) and the remainder were genotype 4 (subgenotype 4c). Many of the HEV isolates from the HEV-positive donors were shown to closely coexist with swine HEV isolates in Hokkaido. Moreover, the ratio of those who consumed organ meat such as pig liver and/or intestines within two months of donation in the HEV-positive donors was higher than that in the ordinary donors (65% vs. 28%, $P < 0.0001$), indicating that the donors infected with swine HEV through zoonotic food-borne routes.
Summary/Conclusions: In Hokkaido, Japan, the frequency of HEV RNA-positive blood donor was 0.038% with ID-NAT. HEV infection may be associated with the consumption of organ meat, particularly from pigs. HEV NAT has contributed greatly to blood safety as no transfusion-transmission cases have been observed in Hokkaido since the implementation of real-time HEV NAT.

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FEASIBILITY OF USING UMBILICAL CORD BLOOD PLASMA FOR DETECTION OF HEV RNA

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Background: Infectious disease screening of umbilical cord blood (UCB) donations is usually performed on a blood sample obtained from the infant's mother. However, maternal plasma may not be available for performing additional testing of UCB products that are in frozen storage. UCB specimens have been reported to have significant amounts of inhibitors in plasma. Routine DNA extraction may not be sufficient to remove all of these inhibitors and therefore may result in false negative or low-level reactivity in polymerase chain reaction (PCR) tests.

Aims: A study was performed to explore the feasibility of using UCB plasma collected in citrate-phosphate-dextrose (CPD) anti-coagulant as an alternative sample type for the **cobas**® HEV nucleic acid test for use with the **cobas**® 8800 System (**cobas**® HEV).
Methods: One aliquot from each of 20 paired maternal plasma and CPD UCB plasma specimens was spiked with a HEV secondary standard at a concentration approximately 10× the limit of detection (LOD) for the **cobas**® HEV test. A second aliquot from each of the same 20 paired maternal plasma and UCB plasma specimens were also tested un-spiked. Each aliquot was tested in a single replicate with the **cobas**® HEV test. The **cobas**® HEV test includes an armored RNA internal control (IC) that is processed with the sample and serves as an internal process control during sample preparation, amplification and detection. Reactivity and cycle threshold (Ct) values for the target and IC were compared for maternal vs UCB samples.
Within each pair of specimens, if either the maternal or UCB plasma spiked sample yielded an invalid IC or target not detected for HEV, both specimens would be retested after a 1:6 dilution using HEV negative EDTA human plasma to explore whether inhibitory substances were present in the sample type.
Results: Within each sample type (maternal or UCB) no significant differences were found in Ct values for the IC for spiked vs un-spiked samples, overall for maternal and cord ($P = 0.60$); maternal only ($P = 0.787$); and cord only ($P = 0.534$). Differences in IC Ct values between maternal and cord samples were statistically significant for both spiked ($P = 0.009$) and un-spiked samples ($P = 0.001$). A significant difference ($P < 0.0001$) was observed for HEV Ct values between spiked maternal vs spiked cord samples. The median HEV ΔCt (Cord-Maternal) was 0.81 Ct. In general, HEV Ct values for cord specimens are higher, which suggest inhibitors were present in the cord specimens. All spiked samples were detected.
Summary/Conclusions: Statistically significant differences were noted when the IC Ct was compared between maternal and cord for either spiked or un-spiked samples and statistical significance was observed for HEV Ct values between spiked maternal and spiked cord samples. The study suggests that the presence of inhibitors in the cord specimens resulted in a mild delay in HEV Ct values compared to maternal specimens, although the median HEV ΔCt was less than 1 Ct which may not be clinically important. The mild inhibition did not prevent detection of HEV at 10× LoD.

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THE UTILITY OF COMBINED ANTIBODY TESTING FOR HEPATITIS B CORE AND SURFACE ANTIGEN AS FIRST LINE SCREENING STRATEGY AND RE-EVALUATION OF SELECTED DONORS AT THE SOUTH AFRICAN NATIONAL BLOOD SERVICE

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Background: The South African National Blood Service (SANBS) tests all donations for Hepatitis B virus (HBV) using Individual Donation Nucleic Acid Testing (ID-NAT) and Hepatitis B Surface antigen (HBsAg). Testing for antibodies to Hepatitis B core antigen (Anti-HBc) is not performed as a first line test as it has been indicated that as much as 70% of the South African population are exposed to Hepatitis B in their lifetime. Currently, Anti-HBc is used as a confirmatory test for HBV DNA+/HBsAg- donations and as a supplementary test for donors who previously tested NAT non repeat reactive or who have history of hepatitis. Anti-HBc+ donations are made available for patient use provided the anti-HBs titre exceeds 100 IU/ml and HBsAg and NAT are negative. Anti-HBc is performed as a first line test in some countries which adds a safety margin by excluding occult hepatitis B donors.
Aims: The aim of this study is to determine the rate of anti-HBc+, anti-HBs+ and anti-HBs titre levels in NAT-/HBsAg-donors. In addition it aims to assess initial HBV NAT+, HBsAg- returning donors and the impact of re-instating such donors who test anti-HBc and anti-HBs only positive (titre >100 IU/ml) on a follow up sample.
Methods: A cross-sectional study of 3446 NAT- (Ultrio Plus, Grifols) /HBsAg- (Prism HBsAg, Abbott) donations for anti-HBc (Cobas, Roche) to determine anti-HBc prevalence. All anti-HBc positive donations with sufficient plasma were tested for anti-HBs titre (Roche Cobas).
Donors who tested HBV DNA+/HBsAg- on their initial donation between 2011 and April 2015 who returned were assessed for NAT, HBsAg, anti-HBc and anti-HBs reactivity.
Results: Of the 3,446 HBV DNA-/HBsAg- donors, 322 tested Anti-HBc positive (9.34%). Anti-HBs reactivity was detected in 203/246 (82.5%) of the anti-HBc positive samples. In 165 of the 246 anti-HBc positive donors the anti-HBs titre exceeded 100 IU/ml (67.1%). The prevalence of an anti-HBs titre >100 IU/ml amongst anti-HBs positives in this group was 81.3% (165/203).
Sixty nine initial HBV DNA+/HBsAg- donors were confirmed on a follow up sample. Of these 35 (50.7%) donors seroconverted to HBsAg. Twenty three (33.3%) tested HBV DNA and HBsAg negative but anti-HBc positive on follow up. In addition all 23 were anti-HBs positive and 11 also tested anti-HBc IgM positive. Of the 12 donors that only tested anti-HBc total positive 11 had an anti-HBs titre of >100 IU/ml (average titre 725 IU/ml).
Summary/Conclusions: In a scenario where first line anti-HBc and anti-HBs universal screening is implemented, an additional 3.08% donations would be discarded resulting in approximately 25,000 units per annum. With limitations in the current donor base and constant blood shortage this is probably still not a viable option.
Currently 15.9% of HBV WP donors who return anti-HBc+, anti-HBs >100 with no other reactive results, are being reinstated as blood donors resulting in about 6 donations per annum. This allows for consistent application of the anti-HBc+/anti-HBs >100 IU/ml re-instatement policy.

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Abstract has been withdrawn.

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EVALUATION AND DIAGNOSTIC EFFECTIVENESS OF TTI SCREENING KITS IN PAKISTAN

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Background: Screening for transfusion-transmissible infections (TTIs) is a critical part of the process of ensuring that transfusion is as safe as possible. Unsafe blood transfusion is very costly both from a human and an economic point of view. In Pakistan, the transfusion-transmitted infections especially HBV and HCV are prevalent and the screening is performed on rapid devices which are not quality assured. The TTI screening kits have never been evaluated and validated at a national level. There is in fact, no national strategy for a testing technique and algorithm, which could have resulted in a consistent quality of screening across the country.
Aims: The current study was undertaken to evaluate the performance and diagnostic effectiveness of commercially available rapid screening kits in comparison with Chemiluminescence Immunoassay (CLIA) and Polymerase Chain Reaction (PCR).
Methods: This single centre, cross sectional study was conducted at the Department of Blood Transfusion Services, Shaheed Zulfiqar Ali Bhutto Medical University, Islamabad, from July 2016 – February 2017. Ten commonly used brands of immunochromatographic (ICT) test kits were used for evaluation and included High Top, Right Sign, Wondfo, Accu-Check, Fast Step, Abon, Immu-Med, Insta-Answer, Biocheck and CTK. 100 positive (including borderline) and 100 negative samples were selected and tested on each of the 10 rapid devices and LIAISON® XL chemiluminescence immunoassay. The Polymerase Chain Reaction was used as a gold standard and performed by ONE-STEP RT-PCR PreMix Kit. The factors considered for the evaluation was sensitivity and specificity, positive and negative predictive values, and accuracy index.
Results: HBV Assays

Statistical Parameters	Rapid Kits										
	High Top	Right Sign	Wondfo	Accu-Check	Fast Step	Abon	Immu-Med	Insta-Answer	Biocheck	CTK	Liaison-XL
Sensitivity, %	65	67	62	70	68	73	77	80	67	72	100
Specificity, %	70	85	73	80	77	85	83	90	81	83	98
Positive Predictive Value	0.68	0.81	0.70	0.77	0.75	0.82	0.82	0.89	0.78	0.81	0.98
Negative Predictive Value	0.66	0.72	0.66	0.72	0.70	0.75	0.78	0.82	0.71	0.74	1
Accuracy Index, %	67.50	76.0	67.50	75	72.50	79.0	80.0	85.0	74.0	77.50	98.5

HCV Assays

Statistical Parameters	Rapid Kits										
	High Top	Right Sign	Wondfo	Accu-Check	Fast Step	Abon	Immu-Med	Insta-Answer	Biocheck	CTK	Liaison- XL
Sensitivity, %	69	76	69	78	68	63	71	79	62	69	100
Specificity, %	80	83	81	79	68	73	70	68	66	78	98
Positive Predictive Value	0.77	0.81	0.78	0.78	0.68	0.70	0.70	0.71	0.64	0.76	0.98
Negative Predictive Value	0.72	0.77	0.72	0.78	0.68	0.66	0.70	0.76	0.63	0.71	1
Accuracy Index, %	75	79	71	78	68	68	70	73	64	73	99.0

Summary/Conclusions: The evaluation of kits on such a larger scale has never been achieved. The data generated will support the policy makers to prepare future plan of action and introduce the concept of quality and good governance in blood centres.

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DETECTION OF THREE BLOOD DONORS WITH MULTIPLE MYELOMA BY ROUTINE ID-NAT SCREENING

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Background: ID-NAT screening of all donations from voluntary non-remunerated blood donors (VNRD) in Croatia was introduced in 2013. During the period of 3 years and 5 months 639,688 blood donations were screened. Samples of 3 VNRD caused persistent problems in the NAT testing due to the clots formed during protein denaturation and caused the blockage of the aspiration system on testing devices.
Aims: The aim was to investigate the health status of VNRD whose samples caused blockage of the aspiration system and give invalid testing results.
Methods: ID-NAT screening was performed using the Procleix Ultrio Plus assay on the three Tigris devices (Grifols, Spain) that is designed to simultaneously detect genomes of three viruses: HBV, HCV and HIV-1 in multiplex test. According to the manufacturer of the test, higher rate of invalid results may be observed in serious clinical conditions associated with high plasma protein content (diseases with positive rheumatoid factor or positive ANA, systemic lupus erythematosus, multiple myeloma, multiple sclerosis, rheumatoid arthritis, hyperglobulinemia, alcoholic cirrhosis). Three donors whose blood sample showed interference in ID-NAT test were invited for an interview and second sample.
Results: Procleix Tigris System declared the results of initial testing of three VNRD as invalid. Interference persisted in repeated testing. VNRD were invited to CITM to take targeted anamnesis and blood samples for additional testing: basic coagulation tests, total protein and serum protein electrophoresis. All VNRD were man, aged above 40, considered themselves as healthy people. Two donors had increased sweating several months ago, one was anemic and treated by physiatrist because of the pain in the arm and neck. All donors had normal coagulation tests results and increased amount of total protein (152.0 g/l, 99.4 g/l, 94.5 g/l). All three donors were diagnosed as Myeloma multiplex (MM) IgG lambda, two of them classified as ISS (International Staging System) III, and the third as ISS II. The first donor is presented by hyperviscosity syndrome, increased calcium level, renal dysfunction and destructive bone lesions. He was treated by plasmapheresis, three lines of chemotherapy and autologous peripheral blood stem cell transplantation (PBSCT). Other donor was also treated with three lines of chemotherapy and PBSCT. Both are now on maintenance MM therapy. Third donor has asymptomatic multiple myeloma and he has no indication for treatment according to the guidelines of IMWG (International Myeloma Working Group).
Summary/Conclusions: Coincidentally detection of serious disease during routine NAT screening has enabled transfusion service professionals to take additional care about blood donors. We believe that our experience will be helpful to other colleagues working on NAT testing in drawing attention to this important finding.

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EPIDEMIOLOGICAL TRENDS IN HCV, HBV AND HIV SEROLOGICAL MARKER DETECTION IN POLISH BLOOD DONORS (2005–2015)

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Background: An integral part of hemovigillance is the epidemiological analysis of blood borne infectious agents in blood donors.
Aims: Analysis of epidemiological data from serological screening of Polish blood donors for hepatitis B virus (HBV), hepatitis C virus (HCV) and human immunodeficiency virus (HIV1/2) in the period 2005–2015.
Methods: Screening was performed in 23 Polish blood transfusion centers (BTCs) in 2005–2015. The study included test results for 12,513,283 donations collected from 6,408,819 donors. Screening data for anti-HCV, HBsAg and anti-HIV or anti-HIV/p24 were analyzed. All repeat reactive results confirmed in verification tests (NAT and/or WB or neutralization test) were regarded as positive.
Results: The cumulative prevalence (95% confidence interval – CI) of HBV, HCV and HIV positives per 100,000 first time donors, amounted to 456.8 (448.1–465.5), 287.7 (280.8–295) and 8.35 (7.16–9.53); the incidence rate was calculated for 1.67 (1.28–2.07), 11.4 (10.4–12.5) and 5.9 (5.15–6.64) per 100,000 repeat donors respectively. The analyzed infections were detected more frequently in first time donors

($P < 0.05$); the relative risk (RR) of infection in this group as compared to repeat donors for HBV and HCV was 272.8 (215.3–345.7), and 25.06 (22.83–27.52) respectively and for HIV only 1.42 (1.17–1.71). For all viruses the rates were higher in men than women ($P < 0.05$) – RR (95% CI) for the HBV was 1.36 (1.29–1.43), for HCV 1.18 (1.12–1.24) and 3.60 for HIV (2.56–5.04). HIV frequency for all donors ranged from 4.75 in 2007 to 9.36/100,000 donors in 2009. Nevertheless, an upward trend was observed for the 11 years. Until 2008 the frequency of HCV infections increased (from 69 to 162/100,000 donors), then decreased (to 49/100,000 donors in 2015). Steady decrease in HBV infection rate was apparent (from 264.5 to 53.1/100,000 donors).

For HBV and HCV the highest infection frequency was observed in the age group ≤20, for HIV in the group 21–30. The infection frequency (per 100,000 donors) showed geographical variation. HBV infections were most frequent in BTCs in Lodz (351), Kalisz (231) and Opole (222); HCV in Kielce (209), Lodz (207) and Bydgoszcz (172), and HIV in BTCs in Katowice (11.9), Wroclaw (11.4) and Walbrzych (10.5).HBV infections were the least frequent in Raciborz (104), Lublin (88) and Rzeszow (52); HCV in BTCs in Slupsk (48), Rzeszow (46) and Gdansk (58) and HIV in Bialystok (3.6), Lublin (3.5) and Radom (3.2).

Summary/Conclusions: The highest cumulative infection frequency was observed for HBV, the lowest for HIV. In the years 2005–2015 a decrease in the frequency rate for HBV infections was observed. Since 2009, the detection rate for HCV infections gradually decreased. An upward trend for HIV was reported. All the viral infections were detected more frequently in first-time donors than repeat donors; the slightest differences were reported for HIV. Infections were more common in men than women.

The results will be helpful for estimating the residual risk and development of further strategies to reduce the risk for transfusion borne infections.

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ROBUSTNESS OF THE ABBOTT PRISM METHODS TO BIOTIN INTERFERENCE

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Background: The use of biotin as a supplement has increased significantly in recent years and many health care professionals do not realize their patients are taking high doses. The increase has resulted in an increased prevalence of people being exposed to levels much higher than the recommended daily dose and as a consequence, inaccurate lab results for assays that utilize the free capture biotin-streptavidin methodology. After a comprehensive review of Abbott's current on market PRISM methods, no assays were identified that utilize the free capture biotin-streptavidin; however, 3 assays were identified for subsequent interference testing as they contain streptavidin or biotin in the assay design.

Aims: The purpose of this study was to identify any Abbott PRISM assays that may be susceptible to biotin interference based on assay design and then evaluate the performance of these assays with high concentrations of biotin.

Methods: For each of the 3 PRISM assays: (HIV O Plus; HTLV I/II; and HCV) sample pools were created and spiked with concentrations of biotin between 30–1,000 ng/ml. Two sample pools, one negative and one positive, were used for testing. The biotin spiked samples were tested against a control sample preparation to determine if there was a statistical difference between the untreated and biotin containing specimens.

Results: When the negative sample pools were spiked with increasing concentrations of biotin, the mean S/CO difference was 0.00 for PRISM HIV (LN 3L68), HCV (LN 6D18) and HTLV (LN 6E50). For the positive sample pools, biotin spiking resulted in mean S/CO differences of −0.01 to 0.04 for HIV (LN 3L68), 0.01 to 0.06 for HCV (LN 6D18) and −0.02 to 0.04 for HTLV (LN 6E50), respectively.

Summary/Conclusions: 3 Abbott PRISM assays potentially susceptible to biotin interference were tested at increasingly high concentrations of biotin. None of the PRISM assay showed susceptibility to biotin interference at concentrations up to 1,000 ng/ml.

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SPECIFICITY OF AUTOMATED BLOOD SCREENING ASSAYS DEVELOPED FOR THE NEW ABBOTT ALINITY S SYSTEM

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Background: While serological assays for blood and plasma screening must have a high sensitivity, specificity of the assays is equally important. When blood and plasma centers consider switching to a new method, they are well aware that this may result in a temporary or permanent deferral of donors if the new method is less specific than the previous method that was used for donor testing. This is a major concern especially for those centers that have a very high proportion of multiple time whole blood or plasma donors.

Aims: To evaluate the initial (IRR) and repeat reactive rate (RRR), and specificity of the prototype assays developed for the new Abbott Alinity s System at two sites in Europe using routine surplus samples from whole blood donors (serum or plasma). Initially reactive samples are defined as those that did not test reactive upon repeat testing, whereas the rate of repeatedly reactive samples is the percentage of samples that are repeatedly reactive but not found positive by confirmatory testing.

Methods: Serum and plasma samples from routine whole blood donors were tested by commercially available methods and the following prototype Alinity s assays: HIV Ag/Ab Combo (n = 1,856), HBsAg (n = 1,925), Anti-HCV (n = 1,924), Anti-HBc (n = 1,857), Syphilis (n = 2,708) and HTLV I/II (n = 2,543). Initial reactive results and any samples with a discrepant result to the routine method were retested in duplicate and subject to confirmatory testing.

Results: For the Alinity s HBsAg, Anti-HCV and Syphilis assays, all samples tested initially non-reactive, and the specificity of these assays was determined to be 100%. The Alinity s HIV Ag/Ab Combo assay had one initially reactive sample that turned negative upon retesting in duplicate, for a specificity of 100%. Nine samples were found repeatedly reactive upon testing with Alinity s Anti-HBc, all of which were confirmed positive upon subsequent testing. For Alinity s HTLV I/II, two samples were initially and repeatedly reactive but negative by confirmatory testing, therefore reducing the specificity of this test to 99.92%.

Summary/Conclusions: The prototype screening assays developed for use with the new Alinity s System showed very good specificity when using samples from populations with a high percentage of multiple time donors. These data indicate that the introduction of the newly developed Alinity s screening assays will not cause unnecessary donor deferral, not even temporarily.

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PREVALENCE OF SEROLOGIC MARKERS AMONG BLOOD DONORS WHO USE CONFIDENTIAL UNIT EXCLUSION (CUE) IN KURDISTAN PROVINCE, IRAN

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Background: The Confidential unit exclusion (CUE) option has been used to increase blood safety at blood transfusion.

Aims: This study aimed to compare the results of serologic markers between blood donors CUE-positive ("should not use") and negative ("can be used").

Methods: This cross-sectional study was done at the Kurdistan Blood Transfusion Center, Iran, between 2011 and 2014. Serologic tests were performed using commercial products to detect surface antigens of the hepatitis B virus (HBsAg), antibodies against the hepatitis C virus (anti-HCV) and antibodies against the human immunodeficiency virus (anti-HIV). The seropositive results were confirmed using the confirmatory assays.

Results: Of the qualified donors, 98,847 donors (91,180 male and 7,667 female) during 2011 and 2014 gave blood; 30,088 (30.4%) donations were from first-time and 68,759 (69.6%) donations were from repeat donors. The CUE option was chosen by 918 (26 female and 892 male) donors. Out of this number, 535 (58.2%) were first time donors and 383 (41. 8%) repeat donors. The prevalence of confirmed HBs Ag was 0.5% (5/918) and 0.2% (207/97,929) among CUE-positive and negative donations, respectively ($P = 0.03$). The prevalence of confirmed anti-HCV was 0.76% (7/

918) and 0.04% (47/97,929) among CUE-positive and negative, respectively ($P < 0.001$). The prevalence of confirmed anti-HIV was 0.1% (1/918) and 0.008% (8/97,929) among CUE-positive and negative donations, respectively ($P < 0.01$).

Summary/Conclusions: Because of the higher prevalence of serologic marker positivity in donors who chose the CUE option, offering CUE to blood donors could be a potentially useful method for improving blood safety.

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EVALUATION STUDY OF ROCHE ELECSYS ASSAYS IN BLOOD DONOR SCREENING IN SPAIN (NAVARRA)

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Background: Blood banks in Spain perform screening tests for HBsAg, anti-HCV and HIV Ag/Ab and Syphilis for all donated blood units. In the Blood and Tissue Bank of Navarra an evaluation of screening assays on Roche cobas e602 in comparison with the Abbott Architect was carried out in Mai/June 2016.

Aims: The aim of this study was to evaluate the specificity of Roche Elecsys assays on a fully automated analyzer cobas e602 on blood donor specimens in parallel with our routine system. Also usability of the assays should be evaluated for their possible application in routine.

Methods: The specificity was evaluated on 2,059 specimens from unselected blood donors routinely screened on Abbott Architect (HBsAg Qualitative II, HIV Ag/Ab Combo, Anti-HCV, Syphilis TP). The samples were retested on the same or the next day on cobas e602 (Elecsys® HBsAg II, Elecsys® HIV combi PT, Elecsys® Anti-HCV, Elecsys® Syphilis). Initially positive results were repeated in duplicate. If still positive the result was confirmed by testing material from the blood bag. Discrepant results were further confirmed by NAT result.

Results: Based on the results from testing 2,059 blood donations, the observed specificity of Roche Elecsys assays on cobas e602 and Abbott Architect are comparable.

Specificities of the Roche Elecsys HBsAg, HIV, HCV and Syphilis assays were estimated 100%, 99.76%, 99.90% and 99.85% respectively.

Summary/Conclusions: The observed performance of Roche Elecsys assays on cobas e602 is comparable to Abbott Architect assays in blood donor screening. Time of testing is appropriate for our practice. Calibrators have to be resolved and 100/tests per reagent require a major turn-over of reagents. Retesting of initially positive samples is not automatically performed. Maintenance is easy.

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VALUE OF TREND CONTROLS TO DEMONSTRATE VARIATION IN NUCLEIC ACID AMPLIFICATION TECHNOLOGY (NAT) REAGENT BATCHES AND INSTRUMENTS

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Background: Run controls are functional when they are capable of monitoring the analytical sensitivity of NAT blood screening assays. The optimal concentrations of run and trend controls were established in analytical sensitivity studies of Ultrio versions on dilutions of inactivated standards calibrated in copies/ml.

Aims: We used trend controls of 25 copies/ml to compare the analytical sensitivity of consecutive Ultrio Plus and Elite reagent batches. A statistical method was developed and evaluated for identification of aberrant trends. By such trend analysis NAT users can be alerted before NAT reagent batches with impaired analytical sensitivity are used for blood screening.

Methods: Analytical sensitivity was established by testing HBV, HCV, HIV standard dilution series in Ultrio, Ultrio Plus and Ultrio Elite and detection limits were calculated by probit analysis. Subsequently run control levels at 99.5% hit rate (125 copies/ml) and around 95% reactivity (25 copies/ml) were defined. These so called run and trend controls were evaluated in two national blood centers.

We compared the proportions negative, weak (dynamic) reactive and saturated reactive results between NAT reagent batches and evaluated trends based on Gumbel distribution of S/CO values. Trending using Gumbel distribution on NAT response values can be monitored by median – average S/CO; Δ.

Results: Using trend controls significant differences were established between NAT reagent batches and NAT instruments (data not shown), and over time by comparing the proportions reactives or Δ.

Table Proportion of reactive results of consecutive reagent batches of Ultrio versions on P0067 ViraQ HCV Trend 25 Controls.

Ultrio reagent batch	Trend Control Batch	Proportion reactive	Difference vs all (95% C.I)
Ultrio 1	B4062-001	95.8% (n = 192)	3.4 (2.8, 4.1)%
Ultrio		78.4% (n = 74)	−10.6 (−19.0, −9.1)%
Plus 1	B4062-001 All	91.0% (n = 266)	−1.4 (−2.6, −0.3)%
	B4062-002	88.0% (n = 100)	−4.4 (−6.8, −2.0)%
	Ultrio Plus 2	88.1% (n = 286)	−4.3 (−5.7, −2.9)%
	B4062-002 All	88.3%(n = 386)	−4.1 (−5.6, −3.1)%
	B4062-003	100% (n = 80)	7.6 (7.3, 7.9)%
	Ultrio Plus 3	93.9%(n = 180)	1.5 (0.5, 2.4)%
	Ultrio Plus 4	96. % (n = 203)	3.7 (3.0, 4.3)%
	Ultrio Plus 5	92.1% (n = 228)	−0.3 (−1.4, 0.8)%
	Ultrio Plus 6	92.2% (n = 90)	−0.2 (−1.8, 1.5)%
	Ultrio Plus 7	94.5% (n = 91)	2.1 (0.9, 3.3)%
	B4062-003 All	94.4% (n = 872)	2.2 (1.5, 2.4)%

Summary/Conclusions: Trend controls are instrumental to identify variations in analytical sensitivity of NAT reagent batches. The Δ in S/CO values can be used as a trending parameter for Ultrio performance (like the normally distributed Ct values in real time PCR).

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PERFORMANCE CHARACTERISTICS OF THE FOUR SCREENING ASSAYS, HBSAG QUALITATIVE II, ANTI-HCV, HIV AG/AB COMBO AND SYPHILIS TP ON ABBOTT'S NEW ALINITY I SYSTEM

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Background: The Alinity s system was specifically developed as the next generation screening system for high throughput and large sample volumes in blood and plasma centers. The new Alinity i system supports screening of blood in settings that do not require very high throughput, also in many European countries testing of whole blood or tissue donors is an integral part of hospital laboratories and requires a different instrumentation.

Aims: The aim of the current study is to evaluate the sensitivity and specificity as key performance characteristics of the four main screening assays, HBsAg Qualitative II, Anti-HCV, HIV Ag/Ab Combo and Syphilis TP, on the newly developed Alinity i system.

Methods: The Alinity i HBsAg Qualitative II, Anti-HCV, HIV Ag/Ab Combo and Syphilis TP assays were tested side by side with the corresponding ARCHITECT assays to compare the sensitivity and specificity on both systems. The analytical sensitivity for the detection of HBsAg, Syphilis and HIV-1 p24 antigen was determined using the corresponding WHO standards.

Results: The specificity of the assays on blood donor specimens on Alinity i were 99.96% (5,108/5,110) for HBsAg Qualitative II, 99.86% (5,116/5,123) for Anti-HCV, 99.93% for HIV Ag/Ab Combo (5,336/5,340) and 99.94 (2,694/2,695) for Syphilis TP, corresponding very well to values found for the corresponding ARCHITECT assays (99.96%, 99.88%, 99.91% and 99.98% for HBsAg Qualitative II, Anti-HCV, HIV Ag/Ab Combo and Syphilis TP, respectively).

The overall sensitivity of the Alinity i HBsAg Qualitative II assay was found to be 100% using 496 known positive samples including different genotypes and mutants. The Alinity i Anti-HCV assay showed also 100% sensitivity, detecting all 459 known positive specimens representing different HCV genotypes and stages of infection, including acute and chronic infections. The sensitivity of the Alinity i HIV Ag/Ab Combo assay was 100% when using a panel of 635 HIV-1 and HIV-2 specimens (432 HIV-1 antibodies, including 43 HIV-1 gO; 115 HIV-2 antibodies; 88 HIV-1 Antigen specimens including 71 viral lysates from different genotypes). The sensitivity on Alinity i Syphilis TP assay was 100% (412/412).

The analytical sensitivity of the Alinity i HBsAg Qualitative II assay were determined to be 19.93 to 20.87 mIU/ml and the HIV Ag/Ab Combo assay exhibited analytical sensitivities from 0.53 IU/ml to 0.74 IU/ml. For Syphilis TP the analytical sensitivity was 0.01 IU/ml as measured against WHO standards 05/122 and 05/132.

Summary/Conclusions: The screening assays HBsAg Qualitative II, Anti-HCV, HIV Ag/Ab Combo and Syphilis TP demonstrated equivalent or better performance in terms of sensitivity and specificity on the Alinity i system compared to the corresponding ARCHITECT system and are therefore suitable for whole blood donor or tissue screening when testing is done at a smaller scale or integrated into hospital laboratories.

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CENTRALIZED NAT TESTING ON HUB AND SPOKE MODEL-DO WE NEED TO REVIEW?

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Background: India is amongst the medium developed countries with a population over 1.25 billion with areas of difficult terrains, landslides and limited healthcare facilities especially in far flung areas. Blood Banking in India is highly fragmented with >2,700 blood banks under various categories (Government/Red Cross/Hospital based/NGO).

Hub and spoke model for NAT (Nucleic Acid Testing) testing is based on centralized testing with one centre as mother centre and other centres as feeding centres. Based on this concept, project was started at multi-speciality private hospital in Delhi/NCR in 2011 as mother centre being first centralized NAT lab in India.

Aims: Review Hub and spoke model for benefits V/S risks: Methods: Project was started at multi-speciality private hospital in Delhi/NCR in 2011 as mother centre being first centralized NAT lab in India. Doctor of blood bank of Hub is in charge and signing authority of complete model. How it runs:

- Unique City based centralized NAT screening centre
- Samples from each site collected daily by surface transport in two shifts: Morning & Afternoon
- Results of Samples received in morning is out by afternoon and by late evening for the samples collected in afternoon. Reactive sample's barcode number sent by SMS immediately followed by electronic report & hard copy of signed report submitted to each blood bank by next day

Results: In last 6 years: Samples tested: >2.78 lacs. till date (annual test approx.35k).23 blood centres availed ID-NAT testing services of till date.

2,78,026 samples tested	HIV	HCV	HBV	Co- Infection	Combined Total
NAT Yield#	01	15	164	1	181
Yield Rate	1 in 278,026	1 in 18,535	1 in 1,695	1 in 278,026	1 in 1,536

Summary/Conclusions: Key benefits:

1. Helps develop uniform standards for all TTI testing
2. Efficient processing time for releasing blood units
3. Optimization of resources
4. High Throughput
5. Model helps in saving investment cost in terms of space, manpower, instrument cost-maintenance and running cost

Success Story:

Small centres who are not able to run this specialized test at their place are getting benefitted, more and more are adding up. Same model is replicated in other places and even by State Government Pan-India.

Impact of testing:

No reported case of transmission of HIV, HBV or HCV in last 6 years since implementation. Increased confidence in the blood supply.181 potential TTI's detected and intercepted in six years (prevention of a potential 181- 543 cases of infection through different components.

This model is effective in all aspects and should be applied in other testing and donation parameters and may be step towards centralized blood banking in India.

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EVALUATION OF AN IMMUNOASSAY SYSTEM FOR HBSAG, ANTI-HBC, ANTI-HCV, HIV AG/AB AND SYPHILIS SCREENING IN COLOMBIAN BLOOD DONORS

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Background: Screening of blood donations for relevant transfusion transmitted infections is a pillar for transfusion safety. In regions with high prevalence of these infections and a low rate of repeat donors, blood safety relay mostly on screening assays with excellent diagnostic performance. Comparative evaluations for assessing diagnostic performance of new screening tests for infectious diseases are rarely carried out or published in our region. Furthermore, Hispanic ethnicity of blood donors has been associated with a higher rate of false-positive results for different infectious disease markers and across different platforms.

Aims: To evaluate diagnostic performance of the Roche Elecsys HBsAg II, Elecsys anti-HBc, Elecsys HIV combi PT, Elecsy Anti-HCV II and Elecsys Syphilis assays by direct comparison under field conditions with the respective established platform (Abbott Architect) immunoassays: HBsAg Qualitative II, Anti-HBc II, HIV Ag/Ab Combo, Anti-HCV and Syphilis TP.

Methods: Two thousand and twelve consecutive donor serum samples from Colombian Red Cross National Blood Bank in Bogota, were processed in parallel to screen HIV Ag/Ab, Anti-HCV, HBsAg, Anti-HBc and Syphilis by Electro-Chemi-Luminiscence in Roche Cobas e601 and Chemiluminiscence in Abbott Architect i2000. In accordance to the index by each manufacture instruction for use, the results were interpreted as reactive, borderline and non-reactive. Borderline results were considered as reactive for both methods. All repeatedly reactive samples, either concordant or discordant between the two systems, were investigated by confirmatory assays. Positive percent agreement (PPA) and negative percent agreement (NPA) were calculated. To estimate the inter-method differences, Bland and Altman plots were used. Moreover, data allowed the calculation of certain measures of accuracy, restricted to the positive predictive value (PPV), the detection probability (DP) and the false referral probability (FP) for HIV Ag/Ab, Anti-HCV and HBsAg by each platform.

Results: Seventy-nine samples were excluded from the analysis because they did not comply with various pre-analytic requirements (transport time mainly). PPA and NPA were respectively: not determinate (ND) and 99.79% for HIV Ag/Ab, 16.66% and 99.79% for Anti-HCV, 75% and 100% for HBsAg, 84.62% and 99.64% for Anti-HBc and 58.33% and 99.9% for Syphilis. Bland and Altman plots showed that limits of agreement for all assays were small enough to consider both systems equivalent. However, the scatter of the differences markedly increased as the mean sample/cut-off (S/CO) ratio increased above 0.2. For HIV, only FP could be calculated: 0.21% and 0.10%, for Elecsys and Architecht respectively. For HCV, PPV were 0.2 and 1.17, DP 0.05% and 0.05% and FP 0.20% and 0.26%. For HBsAg, PPV were 1 and 0.75, DP 0.16% and 0.16% and FP 0% and 0.05%, for each platform.

Summary/Conclusions: Apart from a lower than expected PPA for HCV and Syphilis, results showed high agreement among evaluated immunoassays. Although Bland and Altman plots showed equivalence, they were noticeably different compared to those reported in previous studies using European donors and similar screening systems. Despite the presence of verification biased sampling, appropriate estimated accuracy measures showed comparable diagnostic performance for both platforms.

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EVALUATION STUDY FOR BLOOD-BORNE INFECTION SCREENING OF ROCHE ELECSYS ASSAYS ON COBAS E 601 IN BLOOD DONORS OF COIMBRA HOSPITAL AND UNIVERSITY CENTER

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Background: Blood banks perform screening tests for hepatitis B surface antigen (HBsAg), hepatitis B core antibodies (HBcAb), hepatitis C antibodies (HCVAb), HIV p24 antigen, HIV antibodies, human T-lymphotropic virus types I and II (HTLV-I/II) and Treponema pallidum antibodies for all donated blood units. The screening sensitivity and specificity are of utmost importance. Before the introduction of a new automated immunoassay platform, an evaluation of different testing systems is recommended. In the Blood and Transfusion Medicine Department of Coimbra Hospital and University Center an evaluation of screening assays on Roche cobas e 601 in comparison with the Abbott Architect $i2000_{SR}$ system was performed.

Aims: The aim of this study was to evaluate the specificity of electrochemiluminescence immunoassay (eCLIA) Roche Elecsys on a fully automated analyzer cobas e 601 on blood donors population in parallel with our routine based chemiluminescence microparticle immunoassay (CMIA) by Architect (Abbott Diagnostics). Sensitivity evaluation was performed with four commercial seroconversion selected panels as well a performance testing for HBV with a HBsAg low titer performance panel was done.
Methods: Specificity was evaluated on 1,230 serum samples from blood donors routinely screened on Abbott Architect i2000sr for HBsAg, HBcAb, HCVAb, HIVAg/Ab 1 + 2, HTLV-I/II and Treponema pallidum antibodies (ARCHITECT HBsAg Qualitative II, ARCHITECT HCVAb, ARCHITECT HBcAb, ARCHITECT HIVAg/Ab Combo, ARCHITECT HTLV I/II and ARCHITECT TP). The samples previously run on Architect platform were also tested on cobas e 601 (Roche Elecsys: HBsAg II, Anti-HBc II, Anti-HCV II, HIV combi PT, HTLV-I/II and Syphilis). Sensitivity was evaluated using four commercial seroconversion panels (SeraCare PHM939 for HBsAg, SeraCare PHV925 for anti-HCV, SeraCare PRB947 for HIVAg/Ab and SeraCare PSS901 (0615-00179 for Syphilis (TP)) and one Performance Panel (SeraCare PHA107(M) (0805-0304) for HBsAg.
Results: Based on the results from testing 1,230 blood donations, specificity was calculated for all repeatedly reactive. Specificity of Roche Elecsys assays on cobas e 601 and Abbott Architect assays are comparable for HIV (100%), HBsAg (100%), Syphilis (100%/100%) and HTLV (100%/100%). Roche cobas e 601 Anti-HCV II results has higher specificity than Abbott Architect (99.76%/99.51%) on a initially reactive basis, but was lower on a repeatedly reactive basis (99.76%/100%). For HBcAb test a correlation of 99.83% was calculated between both systems. Seroconversion Panels: Roche Elecsys Anti-HCV II assay was found more sensitive in comparison with Abbott Architect for HCVAb. The presence of HCVAb was firstly detected 8 (Roche) and 27 (Abbott) days after first bleed. Abbott Architect HBsAg assay found the presence of HBsAg one bleed earlier (3 days after first bleed) than Roche Elecsys HBsAg II (11 days after first bleed). No difference was seen in the other panels.
Summary/Conclusions: The results of Roche Elecsys assays on cobas e 601 are much comparable in sensitivity and specificity to Abbott Architect assays in blood donor screening. These findings suggested that the two immunoassay platforms had a good correlation and the analytical performance and accuracy of the two systems were fairly good. In conclusion, the Roche Elecsys assays on cobas e 601 could represent another reliable assay for blood donor.

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Abstract has been withdrawn.

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SEROLOGY EVALUATION STUDY OF ROCHE ELECSYS ASSAYS ON COBAS E 601 IN BLOOD DONOR SCREENING

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Background: Before the introduction of a new automated testing system, an evaluation of different testing systems is recommended. In the Blood Transfusion and Research Centre of Sharjah an evaluation of screening assays of TTI on Roche cobas e 601 in comparison with the Abbott Architect i2000 system was performed.
Aims: The aim of the study was to evaluate the specificity of Roche Elecsys assays on a fully automated analyzer cobas e 601 on blood donor specimens in comparison to our routine system. The sensitivity study was performed on three commercial seroconversional panels.
Methods: The specificity was evaluated on 2000 plasma specimens from unselected blood donors routinely screened on Architect i2000sr for HBsAg, anti-HCV, anti-HBc, HIVAg/Ab, HTLV I/II and Syphilis. The samples were retested on cobas e 601 (Roche Elecsys HBsAg II, Roche Elecsys Anti-HCV II, HIV Roche Elecsys HIV combi PT, Roche Elecsys HTLV I/II, Roche Elecsys Syphilis). The sensitivity was assessed by testing three commercial seroconversion panels (SeraCare PHM934 for HBsAg, SeraCare PHV925 for HCV and SeraCare PRB947 for HIVAg/Ab).
Results: The observed specificity of Roche Elecsys assays on cobas e 601 and Abbott Architect assays are comparable for HCV (99.9/99.8), HIV (99.6/99.75), HBsAg (99.9/99.8) and HTLV (99.95/99.95). For Syphilis Roche cobas e601 results (99.95), Abbott Architect (99.65). For aHBc results a correlation of 99% was calculated for both systems. Evaluation of three commercial seroconversion panels for HIV, HCV and HBV showed that Roche Elecsys Anti-HCV II assay was more sensitive than Abbott Architect Anti-HCV. The presence of anti-HCV was firstly detected 8 (Roche) and 27 (Abbott) days after first bleed. Positive samples of the HIV and HBV seroconversion panel were equally detected with both systems.
Summary/Conclusions: The observed performance of Roche Elecsys assays on cobas e 601 is comparable to Abbott Architect assays in blood donor screening. Both Roche Elecsys assays on cobas e 601 and Abbott Architect HBsAg, anti-HCV, anti-HBc, HIV Ag/Ab, HTLV and syphilis assay are considered very suitable for blood donor screening with very good specificity and sensitivity.

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Abstract has been withdrawn.

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ONE YEAR EXPERIENCE OF NAT TESTING AT NATIONAL BLOOD CENTER, MYANMAR

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Background: Blood transfusion not only can save lives and improve the life expectancy but also can cause harmful effects to the recipients. Transmission of Transfusion TTI is one of the adverse outcomes of transfusion.
To prevent transmission of TTIs, quality of screening of technique is one of the important measures like donor education, deferral, retainment of repeated donors and quarantine of blood products including look back study of the recipients.
In National Blood Center (NBC), Myanmar, testing of HIV and syphilis have been started in 1995 and that of HBsAg and HCV Ab have been extended in 2003. At that time, the sero-positive rate of HIV, HBV and HCV were 0.71%, 4.20% and 1.60% respectively.
In 2014, testing system was also upgraded to electrochemiluminescence immunoassay (ECLIA) technique from Rapid Diagnostic Testing System. Nucleic Acid Amplification system (NAT) was introduced only at the end of 2014 with the support of Government but only 27% of donations were tested by NAT from January to December 2015.
In 2015, the sero-positivity of HIV, HBV, HCV among blood donor were 0.16%, 1.66% and 0.32%, and that of general population, 0.59%, 6.5% and 2.7% respectively.
Aims: To observe the NAT yield of Hepatitis B virus (HBV), Hepatitis C virus (HCV) and Human Immunodeficiency virus (HIV) in blood donations at National Blood Center, Myanmar.
Methods: In 2015 (Jan to Dec), total 62,923 donations were screened for TTIs and out of ECLIA negative donations, 17,076 (27% of total donations) were tested by NAT. Serological screening using ECLIA method was performed on Roche cobas e601 system to detect HBsAg, HCV Ab and HIV I/II Ag and Ab. Only seronegative donations were subjected to detect HBV DNA, HCV RNA and HIV I/II RNA by NAT using Roche cobas s201 system in minipool of 6.
Results: After NAT testing of sero-negative 17,076 donations, 44 donations were found reactive for HBV DNA, no reactive case for HCV RNA, and only one reactive case for HIV RNA. And so, NAT yield for HBV DNA was 1:388 and 1:17,076 for HIV RNA.
Higher HBV NAT yield was found in our one-year study at NBC, Myanmar, compare to other reports of nearby countries, but for HIV NAT yield, it was consistent with other countries.
We could not do confirmation testing for NAT reactive donations and follow up investigation of such cases were not done.
Summary/Conclusions: To get better screening sensitivity, and to get safer blood products, screening of TTI should be done by individual NAT system; and to check their seroconversion status, further confirmation of NAT reactive donations and follow up investigation of such donors are required.

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Abstract has been withdrawn.

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COMPARISON BETWEEN THE PREVALENCE OF TTIS, COMBINATIONS AND NAT YIELD IN VOLUNTARY NON-REMUNERATED BLOOD DONORS

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Background: Transfusion transmitted infections (TTIs) is a major challenge to the transfusion services all over the world.
The problem of TTIs is directly proportionate to the prevalence of the infection in the blood donor community. Currently, prevention of TTIs depends on proper pre-donation selection of all voluntary non remunerated blood donors. The pre-donation questionnaire is considered the first line of defence against TTIs. NAT (Nucleic Acid Testing) is used for screening of presence of the nucleic acid of the virus either DNA of HBV or RNA of HCV and HIV in the free serological samples.
Aims: To know the different prevalence rate of each parameter of TTIs, combinations and NAT yield.
Methods: The study took place in the Alexandria Regional Blood Transfusion Center (Alex RBTC). It was conducted on all period from August 2016 to January 2017. Screening was done using EIA HBs Ag, HCV- Ab, HIV Ag-Ab, Syphilis Ab and NAT.
Results: Alex RBTC donations were 26,498 in total over 6 months from August (2016) to January (2017).
The total number of repeatedly reactive and confirmed positive donations for:
Hepatitis B surface antigen: 160 of 26,498 (0.01%), hepatitis C virus antibody: 529 of 26,498 (0.02%), human immunodeficiency virus (HIV): 57 of 26,498 (0.002%), Syphilis Treponema Pallidum antibody: 312 of 26,498 (0.012%), NAT yield: 26 of 26,498 (0.001%) Combinations: 22 of 26,498 (0.001%).
Summary/Conclusions: We found that the highest prevalence rate of TTIs is HCV and the lowest one is HIV. For combinations, the highest one is a combination of HCV& Syphilis and the lowest are combinations of HBV & syphilis, HCV, HIV & syphilis and HBV& HIV. NAT is equal prevalence to combinations but it is low (0.001%) and it is concluded that there is a good selection criteria in the pre-donation process.

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MOBILE BLOOD COLLECTION SITES AND THEIR ROLES IN PROVIDING SAFE AND ADEQUATE SUPPLYING (5 YEARS EXPERIENCE)

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Background: Blood transfusion has been recognized as a life-saving therapeutic action. The demand for blood and their products due to aging population, increment in advanced and elective surgery etc. was increased significantly in recent years. So, the main goal of every blood transfusion organization is to supply sufficient and safe blood and blood components. Blood unites were collected at fixed and mobile sites.
Aims: Determination of the role of mobile sites in providing safe blood supply compared with fixed sites.
Methods: This retrospective study was carried out at Khuzestan blood transfusion organization from 2007 to 2012. Some part of the blood collected at mobile sites and fixed sites were compared. The prevalence and trend of major TTIs which includes HIV, HBV and HCV in mobile sites were also evaluated and compared with fixed sites. Statistical comparison was performed using Chi-square test by SPSS 16, $P < 0.05$ was considered as statistically significant.
Results: The total number of blood donations from 2007 to 2012 was 621,117 out of which 531,527 (85.57%) was collected from fixed sites while 89,590 (14.43%) was collected from mobile sites. Trend of donations in different years is fluctuating. However the overall Blood donation index was estimated as 23.8 per 1,000 population. The prevalence of HIV, HBV and HCV in mobile sites donations was 6.69, 318.11 and 118.31, and in fixed sites was 5.45, 187.38 and 94.63 per 100,000 donations respectively. Also HBV prevalence in mobile sites was significantly higher than fixed sites ($P = 0.014$).
Summary/Conclusions: Our finding showed that blood donation index in Khuzestan province is much better when compared with similar socio-economic and neighboring countries but slightly lower than national blood donation index. The allotment of blood units collected by mobile teams is lower than that of national reports. In addition, the prevalence of TTIs in mobile site blood donations was higher than fixed sites.

P-304

Abstract has been withdrawn.

P-305

INCIDENCE OF HEPATITIS B AND C VIRUSES IN PATIENTS WITH BETA-THALASSAEMIA IN ISLAMABAD, PAKISTAN

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Background: Thalassaemias are inherited genetic disorders of haemoglobin. Thalassaemia incidence in Pakistan is on the rise. It is estimated that about 100,000 patients are presently suffering from thalassaemia major, the severe form of the disorder. The patients of β-thalassaemia are dependent upon lifelong blood transfusion. Multiple transfusions expose them to many blood borne diseases, most commonly hepatitis B and C.
Aims: The aim of current study was to determine the prevalence of HBV and HCV infections among thalassaemia major patients in Islamabad to establish more preventive strategies.
Methods: The study was conducted from June to December 2016, at the Thalassaemia Centre, Shaheed Zulfiqar Ali Bhutto Medical University, Islamabad and the Pakistan Thalassaemia Centre, Pakistan Baitul Mal, Islamabad, Pakistan. Data were obtained by clinical testing of 1,440 ß thalassaemia major patients. The confirmatory screening for HBV and HCV was performed through Chemiluminescent Immunoassay (CLIA). The collected data was coded and entered in a data base file. After complete entry, data were transferred to the SPSS software (IBM Corp. 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.). The study subjects voluntarily participated in the study after informed consent. This study was endorsed by the Ethical Review Board of the International Islamic University, Islamabad.
Results: Of the total 1,440 patients studied, 930 (64.6%) were males and 510 (35.4%) were females. The patients age ranged from 1 to 30 years with a mean age of 7.9 years with ±SD 4.5. Among 1,440 patients, 44 patients were positive for HBV (3.05%) while 295 were positive for HCV (20.4%).
Summary/Conclusions: This study showed that β-thalassaemia patients are at a higher risk of contracting HBV and HCV infections. Regulation, oversight and accountability in the donation, screening and transfusion of blood are among the most basic needs of the blood sector and must be addressed immediately.

P-306

Abstract has been withdrawn.

P-307

EVALUATION OF NUCLEIC ACID TESTING (NAT) IN BLOOD DONORS OF NBTC TIRANA

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Background: Albania is a country with a high prevalence of Hepatitis B. This situation is reflected also in our blood donor population where the prevalence of HBsAg is 5.3%. Also other transfusion transmitted infections have a relatively high prevalence (HCV 0.75%, HIV 0.03%, Syphilis 0.21%). This situation dedicated to the fact that most of our donations come from first time donors (90%) and from family replacement donors (75%). We implemented NAT technology for testing blood donations last year, June 2016. It is well known that NAT allows a highly sensitive and specific detection of virus RNA and DNA by shortening the window period and ensuring safer blood transfusions.
Aims: To evaluate the results of applying NAT screening of blood donors for HBV, HCV and HIV.
Methods: All blood collected has been screened with serology for HBsAg, anti-HCV and HIV Ag/Ab (CMIA, Architect 8200 Abbott). Seronegative donations (20,810) were tested with ID NAT by Procleix Ultrio Elite Assay (Grifols Diagnostic Solutions)

on Procleix Panther System. All initial ID-NAT reactive samples were tested in triplicate and then the discriminatory assay for HIV, HBV and HCV was performed.
Results: Total number of seronegative donations tested with NAT was 20,810. There were 109 (0.5%) results ID-NAT initially reactive with serology negative. Among them 77 (0.37%) were confirmed with discrimination. From the discrimination resulted 76 cases HBV DNA positive (NAT yield 1 in 273 donations for HBV DNA) and one case HCV RNA positive. From the 76 cases negative for HBsAg and HBV DNA positive we tested with anti-HBc and anti-HBs only 23 of them. From the 23 cases tested for anti-HBc, 18 resulted positive (7 associated with anti-HBs and 11 only anti-HBc, with no evidence of anti-HBs).
There was only one case of HCV RNA positive donation among 20,810 seronegative donations. This case has been positive for HCV RNA in August 2016. We followed up this donor and we tested with serology and NAT once more in September 2016 where he resulted again HCV RNA positive and serology negative. After that we couldn't trace him again until March 2017. We tested him in March 2017 and he resulted positive for anti-HCV and HCV RNA positive by confirming in this way a clear window period case for HCV.
Summary/Conclusions: There is a very high NAT yield for hepatitis B in our donor population. Our data show a high percentage of OBI that according to several studies might be infectious for immunocompromised patients and not only. NAT has helped in the detection of potentially infectious HBV donations and in the prevention of the potential risk of the transfusion transmitted HBV. Among 20,810 donations tested for HCV RNA it was one donation in confirmed window serological period detected by NAT by preventing in this way transfusion transmitted HCV infection.
Our data clearly show the benefits of ID-NAT donor screening for the improvement of transfusion safety in our country.

P-308

Abstract has been withdrawn.

P-309

ENHANCING BLOOD SAFETY THROUGH PIONEERING NAT TECHNOLOGY IN EASTERN INDIA

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Background: Transfusion-transmitted infections are a major problem associated with blood transfusion. Nucleic acid amplification testing (NAT) is not yet obligatory in India for blood donor screening. The primary benefit of NAT is the ability to reduce residual risk of infections by preventing window period (WP) donations.
Aims: Here we share our experience of screening blood donors by The Roche cobas TaqScreen MPX platform the first of its kind in Eastern India.
Methods: All blood donations between 23 November 2013 and 28 February 2017 and non-reactive for HBsAg, anti-HIV 1 & 2 and anti-HCV by chemiluminescence (CLIA) were included in the study. NAT, for HBV-DNA, HCV-RNA and HIV-RNA in the minipool of six samples was performed using the Roche cobas TaqMan MPX assay. Individual sample of each positive pool was tested subsequently in the same platform. Each positive sample was then sent to referral PCR laboratory for the viral discrimination and quantitation. Sample positive for hepatitis- B virus (HBV) DNA was further screened for anti-HBc antibody & antibody to HBsAg (anti-HBs). All results were documented & recorded as per the SOP.
Results: Of the total 29,599 blood donations during the study period, anti-HCV, HBsAg and anti HIV by CLIA were detected in 176 (0.59%), 201 (0.68%) and 69 (0.23%) donors respectively. A total of 29,153 samples were tested for NAT of which 11 (0.04%) donors were found to be carriers of HBV DNA each with a viral load of <6 IU/ml. One donor (0.003%) was positive for HIV RNA. The NAT yield was observed to be 1 in 2,466 donations. Among the 11 HBV DNA carriers eight were sero-reactive for anti-HBc and six donors developed borderline reactive anti-HBs antibody.
Summary/Conclusions: Introduction of NAT has successfully identified pre-seroconversion infectious blood donors and occult hepatitis B. Despite its cost effectiveness issues NAT will be a standard of blood donor screening in the future. With the implementation of NAT in our blood centre we could save 36 patients who would have otherwise received the infected blood components.

P-310

NUCLEIC ACID TESTING OF BLOOD DONATIONS: RESULTS FROM A TERTIARY CARE HOSPITAL IN INDIA

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Background: Nucleic acid testing (NAT) for detection of HIV 1,2 RNA, HCV RNA, and HBV DNA in blood donations have been implemented for early detection of window period infections and has resulted in reduction of infectious window period donations. Immunoassays detect antibodies to viral antigens, which may miss window period (WP) donations. Occult Hepatitis B infection (OBI) is serologically undetectable hepatitis B surface antigen (HBsAg-ve), despite the presence of circulating HBV DNA and anti-HBc.
Aims: To perform Nucleic acid testing in seronegative blood donations and detect window period donations. This is a descriptive study.
Methods: We performed NAT on Roche cobas s 201 system, MPX test, multidye test, version 2.0, using polymerase chain reaction (PCR), minipool of six donations on 27,916 blood donations (from May 2012 to 8th March 2017), which were seronegative for HBsAg, anti-HCV and anti-HIV by enhanced chemiluminescence immunoassay (ChLIA), on vitros EciQ (Ortho-Clinical Diagnostics, USA). Anti-HBs was done for those donations which were positive for anti-HBc by ChLIA. The MPX v2 test, is a qualitative multiplex test that enables the simultaneous detection and discrimination of HIV RNA, HCV RNA, HBV DNA and an internal control in a single assay.
Results: We identified 18 NAT yield donations, of which 17 were positive for HBV DNA and 1 was positive for HIV RNA. Of the 17 HBV positive donations, 10 donations were positive for anti-HBc and negative for anti-HBs, two donations were positive for anti-HBc and positive for anti-HBs and the remaining five donations were negative for anti-HBc.
Summary/Conclusions: Of 27,916 blood donations, 18 NAT yields were detected; which suggests that 1 in 1,550 donations were found to be NAT reactive. Of 18 NAT yields, one donation was found to be reactive for HIV and 17 donations reactive for HBV. Of 17 donors who were HBV reactive, we detected 10 donors who had OBI and the remaining seven donors were in window period. Multiplex nucleic acid testing detected potentially infectious HBV and HIV during the window period before seroconversion and prevented residual risk of transmission of HBV and HIV, which added additional blood safety.

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P-316

SCREENING DONATED BLOOD FOR TRANSFUSION TRANSMITTED INFECTIONS BY SEROLOGY ALONG WITH NAT: A NAKASERO BLOOD BANK EXPERIENCE

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Background: Blood transfusion safety has tremendously improved through testing of donated blood with increasingly sensitive assays compared to the historical serologic testing for transfusion transmitted diseases. Although newer strategies like nucleic acid testing (NAT) have helped further shorten the "window period" no technology exists to completely detect all window period donations and therefore none completely closes the exposure-to-seroconversion window period.
Aims: To establish the relative safety of blood at which screened using NAT and serology. To establish the rate of false positive on serology.
Methods: 642 donations were screened individually for some TTIs, namely; HIV, HBV and HCV by serology and nucleic acid testing (NAT). All reactive samples were retested (wherever possible). The reactive results of either serology or NAT were followed up, blood units were discarded and donors were notified and counseled.
Results: We evaluated 32(4.98%) cases which were reactive on both NAT and Serology.28 (4.36%) cases were detected on NAT (1.1% HIV, 0.16% HBV, 0.16% HCV). 4 (0.62%) cases were however reactive on serology and non detected on NAT (0.16% HIV, 0.31% HBV and 0.16% HCV).
Summary/Conclusions: Our study indicated that it is important to use both NAT and serology to improve on the blood safety, a practice which is not yet embraced at Uganda Blood Transfusion Service. Screening of blood for TTIs using Serology alone is associated with high rate of false positive and increased donor deferral.

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TTI MARKER PREVALENCE OF THE DONORS IN THE REGIONAL CENTER TETOVO

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Background: One of the main factors for evaluation and monitoring of the donor selection and screening effectiveness, as well as for blood safety, is the surveillance of the prevalence of transfusion transmitted infectious diseases markers in blood donors. According to the Macedonian national legislative the examination is done on HbsAg, HIV, HCV and Treponema Pallidum (TP) antibodies.
Aims: To analyze the seroprevalence of mandatory tested TTIDs markers in blood donors at the Regional Center Tetovo, after the integration of the Center to Institute for Transfusion Medicine Skopje, more precisely from 2011 to 2016.
Methods: 9.616 units has been screened for HbSAg, anti – HIV – combination, HIV – 1/2 antibodies, anti HCV and anti TP using EIA – Enygnost – Siemens and CMIA – Architect System – Abbot at the Institute in Skopje.
Results: The total number of initially reactive samples during the period of 6 years was as follows: 100 (1.03%) for HBV, 23 (0.23%) for HCV, 1 (0.01%) for HIV, 7 (0.07%) for TP. Out of the total 100 HBsAg +, 28 were positively confirmed (0.29%). Out of the total 23.5 HCV+ (0.05%) were positively confirmed. There were no HIV and TP positively confirmed samples.
Summary/Conclusions: After the integration of the Regional Center Tetovo, blood donation for family members has gone into the history, even though it was very common before the integration. In the Republic of Macedonia blood donation is voluntary and non – remunerative. There is a good and safe blood service for the patients, with high quality of monitoring and selecting blood donated by voluntary donors. The obtained results correlate with those on the level of the Institute for transfusion medicine in Skopje.

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IS CHIKUNGUNYA VIRUS A BLOOD TRANSFUSION PROBLEM IN NORTH PORTUGAL?

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Background: Chikungunya virus was first isolated in 1953 by R.W. Ross from a Tanzanian patient with fever and joint pains. Chikungunya (in the Makonde language "that which bends up") virus (CHIKV) is a single-stranded positive sense RNA virus, classified in Group IV, *Alphavirus* genus and *Chikungunya virus species*. Three genotypes of this virus have been described, each with a distinct genotype and antigen character: West African, East/Central/South African and Asian genotypes. CHIKV is transmitted to people through mosquito bites. Mosquitoes become infected when they feed on a person already infected with the virus. Infected mosquitoes can then spread the virus to other people through bites. Chikungunya virus is most often spread to people by *Aedes aegypti* and *Aedes albopictus* mosquitoes. These are the same mosquitoes that transmit dengue virus. Chikungunya virus is transmitted rarely from mother to newborn around the time of birth. In theory, the virus could be spread through a blood transfusion. The incubation period range from3 to 12 days. The onset is usually abrupt and the acute stage is characterized by sudden high fever, incapacitating arthralgia, myalgias and skin rash.
Several methods (serologic and molecular) can be used for diagnosis. The virus may be isolated from the blood during the first few days of infection. The rt-PCR are available and is important at this time of infection. Portugal is closed to the Atlantic Ocean and we have lots of people traveling to endemic areas specially to our ex colonies of Africa, South America and India.
Aims: The aim of this study is to test a group of voluntary blood donors, at time of donation, looking for the presence of RNA CHIKV, to assess the transmission of this virus by transfusion. By the other side, we want to evaluate the RNA- CHIKV test used.
Methods: Nucleic acid extraction NucliSENS easyMag- bioMerieux.
Rotor Gene Q- Qiagen.
Polymerase Chain reaction (PCR-CHIKV): Fast Track Diagnostics (FTD)-Luxembourg.
Quality Control for Molecular Diagnostics (QCMD) -10 samples panel of different concentrations-Scotland, UK (Testing in 2 different runs).
110 whole blood samples recently collected (on day of test running) from voluntary blood donors choosing among high risk travel in the past.
8 patients with epidemiologic or clinical suspicion of infection.
10 samples were repeated, to show the reproducibility of the test.
Results: The positive and negative controls used in all the runs had correct results as the QCMD samples panel tested in the two different runs; The repeated samples were the same results; We did not find any positive results in blood donations or patients.
Summary/Conclusions: We did not detect any positive RNACHIKV, indicating any active infection at time of donation. The FTD CHIKV test seems to be sensitive, reproducible, specific (in the tests carried out on the panel). In parallel, one should search for antibodies but are not currently available in our center. We will do them soon. This is a small, single centered study, thus, further studies are needed to make these results more consistent.

P-319

CHOOSING A MOLECULAR TEST TO SCREEN DENGUE INFECTION

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Background: Dengue virus (DENV) is a positive strand RNA virus of the genus *flavivirus, Flaviviridae* family.
DENV is transmitted to humans mainly by the bite of infected *Aedes* mosquitoes, mostly *Aedes aegypti* and can be also transmitted by blood transfusion and transplantation of organs and tissues.
DENV infection is a major cause of disease in tropical and subtropical areas, with 50–100 million infections occurring each year. DENV is the only human arbovirose (viral disease transmitted to man by arthropod vectors) that can be caused by four different viruses since any of the serotypes (DENV1, DENV 2, DENV3 and DENV4) is capable of causing infection.

After an incubation period of 2–7 days, dengue infection becomes asymptomatic in the majority of cases. But it may result in a wide spectrum of clinical symptoms, ranging from a mild flu-like syndrome with fever in combination with severe headache, myalgia and arthralgia, known as "dengue fever" to the most severe forms of the disease, which are characterized by leucopenia, thrombocytopenia, increased vascular fragility and permeability (dengue hemorrhagic fever) and may progress to hypovolemic shock (dengue shock syndrome).
Portugal is in the crosspoint between Europe, Africa and America, with many blood donors coming or traveling from Brazil and Madeira Island where this virus is endemic.
Aims: The aim of this study is to test a group of voluntary blood donors, at time of donation, looking for the presence of RNA DENV, to assess the transmission of this virus by transfusion. By the other side, we want to evaluate the RNA- DENV test used.
Methods: Nucleic acid extraction NucliSENS easyMag- bioMerieux. Rotor Gene Q-Qiagen. Polymerase Chain reaction (PCR-DENV): Fast Track Diagnostics (FTD)-Luxembourg. Quality Control for Molecular Diagnostics (QCMD) -10 samples panel of different concentrations-Scotland, UK (Testing in two different runs). 110 whole blood samples recently collected (on day of test running) from voluntary blood donors choosing among high risk travel in the past. Eight patients with epidemiologic or clinical suspicion of Dengue infection. 10 samples were repeated, to show the reproducibility of the test.
Results: The positive and negative controls used in all the runs had correct results. The QCMD samples panel tested in the two different runs were all correct results. The repeated samples were the same result in both runs. We did not find any positive results in blood donor samples nor patients.
Summary/Conclusions: The FTD DENV test seems to be sensitive, specific (in the samples panel used) and have good reproducibility.
We did not detect any positive RNA DENV, indicating we did not have any active infection on the samples tested.
This is a small, single centered study, thus, further studies are needed to make these results more consistent.

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WHAT ABOUT WEST NILE VIRUS INFECTION IN NORTH PORTUGAL?

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Background: The West Nile virus (WNV) is one of the most important and pathogenic of the many members of the genus *Flavivirus* that are known to cause human disease especially West Nile Fever and neuroinvasive illness. The WNV causes serious manifestations in approximately 1% of person who are infected. The life cycle of the West Nile virus involves the transmission from nonhuman animals to humans by way of *Aeds, Culex or Anopheles* mosquitoes. The West Nile Virus can infect horses, birds, dogs and other mammals, although wild birds seems to be the optimal hosts for harboring and replicating the virus. WNV presence in Portugal is emerging, with sporadic cases of infection in horses and humans. In 2010 a human case was identified in southern Portugal. Several case report documented transmission through blood transfusion, transplant organs, breast feeding and vertical transmission. Efforts have been made in an attempt to obtain accurate diagnostic tests, to search for an effective therapy and to control the vectors. Viral cultures and tests to detect viral RNA (reverse transcriptase-polymerase chain reaction – rt PCR) can be performed on serum, cerebrospinal fluid and tissue specimens that are collected early in the course of illness and, if results are positive, can confirm an infection (CDC-Center for Disease Control). Immunoassays for WNV specific IgM and IgG are also available.
Aims: The aim of this study is to test a group of voluntary blood donors, at time of donation, looking for the presence of RNA WNV, to assess the transmission of this virus by transfusion. Additionally we want to evaluate the rt PCR used to search RNA- WNV.
Methods: Nucleic acid extraction NucliSENS easyMag- bioMerieux. Rotor Gene Q-Qiagen. -Real Time Polymerase Chain Reaction qualitative detection of WNV RNA Sacace Biotechnologies- Itália (CE marking). Quality Control for Molecular Diagnostics (QCMD). 10 samples panel of different concentrations-Scotland, UK. (Testing in 2 different runs). 160 plasma samples from voluntary blood donors. Three patients who live in the south of Portugal. 10 samples were repeated, to show the reproducibility of the test. In each run we placed 1 external positive control, 1 external negative control, 1 amplification positive control and 1 amplification negative control.
Results: All the controls used presented correct results. The QCMD samples panel tested in the two different runs presented correct results. The repeated samples had the same results. We did not find any positive results in blood donor samples nor patients.
Summary/Conclusions: The Sacace rt-PCR to test WNV RNA seems to be sensitive, specific (looking at the results of the panel samples) and have good reproducibility. We did not detect any positive WNV RNA, indicating we did not have any active infection in blood donations tested. This is a small sample size. A more extensive study should start soon.

P-321

COMPARISON BETWEEN TRENDS OF TRANSFUSION TRANSMITTED INFECTIONS IN TRANSFUSION DEPARTMENT STRUMICA IN PERIOD OF 2015 AND 2016 YEAR

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Background: Blood transfusion can be a life saving procedure. Transfusion transmitted infection is any infection that is transmissible from blood donor to a patient through blood or blood product. But testing for TTI with sensitive screening tests is the final measure for eliminates unsafe blood. Avoiding family donors, selecting donors through questionnaires and regular doctor's examination and limiting the number of blood transfusions can ensure the elimination, or at least reduction of the risk of acquiring transfusion transmitted infections.
Aims: To estimate the risk of transfusion transmitted infection in blood donors in Transfusion center Strumica in period from 01 January 2015 year till 31 December 2015 year and compare this risk with the same in period from 01 January 2016 till 31 December 2016.
Methods: Our study is an comparison between two year retrospective study with blood banking data from our registers and include all donors who were screened for TTI by using immunological methods-enzyme imunoabsorbent assay ELISA for anti-HIV 1,2, Anti-HCV, HbsAg and Syphilis.
Results: Total number of donated blood units in 2015 year was 1976. 1900 of them (96%) were voluntary blood donors, 73(4%) were family. Males are majority of donor population-1584 (80%), females are 389 (20%). First time donors are 267 (13%), 1,709 (87%) are regular donors. In that period of one year, initial reactive blood donors were: HbsAg-35(1.78%), antiHCV-2(0.1%),AntiHIV-2 (0.1%) and Syphilis- 1(0.05%). 30 of them were retested (from new sample of blood) and the repeat reactive blood donors were confirmatory tested. Confirmatory test (for HBs Ag) was positive in only four blood donors (0.2%). Two of them were first time donors, two were regular donors. Initially reactive blood donors for anti-HCV and anti-HIV were invited by letter for a new sample blood, but they did not answer the invite. Total number of donated blood units in 2016 year was 1902. 1,227 of them (96%) were voluntary donors, 72 (or 4%) were family. Males are 1,539 (81% from total number of blood units), females are363 (19%). First time donors in 2016 year are 297 (15.6%), regular are 1,605 (84.4%). In 2016 year, initial reactive blood donors were: HbsAg- 8 (0.4%), antiHCV-1(0.05%), anti-HIV -2 (0.1%) and Syphilis -1 (0.05%).7 of them were retested (from new sample of blood) and the repeated blood donors (2 regular- not first time donors, one for anti-HIV, one for anti-HCV)-were confirmatory tested in February 2017 year, but we still do not have the results from our referent center for confirmation.
Summary/Conclusions: Our study documents low percentage of TTI among our blood donors, high percentage of voluntary donors, low participation of female donors and no difference between TTI in first time and regular donors in the studied 2015 year. In 2016 year – our study documents some changes: low increase of percentage of first time donors but decrease the percentage of initial reactive blood units, especially for HbsAg (versus our expectance- a larger number of first time donors- a larger number of blood units with tti). The target for transfusion specialists should be provision strict criteria in recruitment and deferral of blood donors, proper testing of every blood units by using recommended standard methods how we can improve safe transfusion.

Hepatitis B (HBV)

P-322

Abstract has been withdrawn.

P-323

THE STUDY OF SEROLOGICAL AND VIROLOGICAL FEATURES OF OCCULT HBV INFECTION IN SOUTHERN CHINA

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Background: The Nucleic Acid Test (NAT) of blood donations has been implemented nationwide since 2015 in China. The current blood screening strategy of both Enzyme Immunoassays (EIAs) and NAT contribute to the detection of most pre-seroconversion window period infection (WPI) and occult HBV infection (OBI), It also benefits the research of OBI, which have not been evaluated extensively among Chinese population, especially in large samples.
Aims: This study is to investigate the incidence and the serological and virological features including HBsAg, HBV DNA, anti-HBc and anti-HBs of OBI for further understanding the risk of transfusion transmitted HBV infection.
Methods: 52,679 voluntary donors were recruited by Guangzhou blood center between January 1 and August 31, 2015. All the blood donations were screened by two EIAs and the Ultrio NAT for pathogens. Following exclusion of donors positive for HCV, HIV, TP markers or with elevated ALT and triplex NAT, 52,024 donors were recruited for this study. HBsAg-/DNA+ donors were further tested HBV DNA load by Q-PCR and S region amplification by nested-PCR and then sequenced; anti-HBc were detected by two different EIAs and quantitative anti-HBs were determined by electrochemiluminescence immunoassay. All the HBsAg-/DNA+ donors were followed up and the follow-up blood samples were retested by serological and virological methods as mentioned above.
Results: The incidence of OBI in blood donors in Southern China was 1:1,156, consisting of 1:1,060 in first-time donors and 1:1,494 in repeat donors. 52.93% OBI donors had HBV DNA load 0–49 IU/ml, 13.73% was 50–99 IU/ml, 9.81% was 100–199 IU/ml and 23.53% with the DNA load higher than 200 IU/ml. The retesting of follow-up samples showed fluctuating level of DNA, especially in those samples with DNA load lower than 100 IU/ml. The positive rate of anti-HBc in OBI donors was 91.12%. Among them, 42.23% were anti-HBc+, 48.89% were both anti-HBc+ and anti-HBs+; anti-HBs+/anti-HBc- and anti-HBc-/anti-HBs- were both 4.44%. The titer of anti-HBs was under 10 IU/L in 56% OBI donors, 10–100 IU/L in 36% OBI donors and 8% donors has higher anti-HBs above 100 IU/L. Most of OBI (92.31%) we found in this study were HBV B or C wild type. Only 1clone of 1 donor had a mutation (S124C) in MHR of S region.
Summary/Conclusions: NAT decreased the risk of transfusion transmitted HBV infection, however, because of high prevalence of HBV in China and the absence of anti-HBc screening, OBI remains a continual risk factor of transfusion transmitted HBV. Implementation of more sensitive NAT in blood screening could reduce HBV by blood transfusion. Further research is needed to reveal the mechanism of the mutations lead to OBI in Chinese population.

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BLOOD SAFETY POLICY DECISION-MAKING FOR OCCULT HEPATITIS B IN GREECE

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Background: Nucleic acid testing (NAT) can reveal Occult HBV infection (OBI) in donor blood. OBI is a form of chronic HBV infection characterized by undetectable HBsAg and very low levels of HBVDNA with or without anti-HBc or anti-HBs antibodies outside the preseroconversion window period. Previous studies in Greece have shown high prevalence of OBI in donor blood (1/6,080 units) (Politis et al. Vox Sang.93:2007). HBV DNA load was below 351 IU/ml in all 21 OBI cases due to mutations in the RT region, 57.1% were anti-HBc positive and 42.9% were positive for both anti-HBc and anti-HBs. The diagnostic failure of the HBsAg assays could possibly be explained by the multiple mutations in the S gene of the Greek blood donors (Katsoulidou et al. JMV 81:2009).
Aims: The aim of this study is to reexamine the epidemiological features of OBI in the Greek donor population, to refine our strategy for the management of donors with true OBI and to obtain risk estimates from undefined and inconclusive cases.
Methods: In 2015 we tested 520,844 blood units for HBsAg with standard serological assays (MEIA, CHLIA). Blood samples were also screened individually by a triplex HIV/HCV/HBV NAT assay (PROCLEIX ULTRIO Multiplex Assay and S201 COBAS TaqScreen MPX). Initially or repeatable NAT HIV/HCV/HBV reactive samples were further retested using the discriminatory assay to identify the exact viral infection. In all of the NATHBV yield samples, dHBV discriminatory assay was performed. Anti-HBc and anti-HBs were examined in all HBsAg negative/NATHBV positive samples. Anti-HBe was examined if required.
Results: Among NAT HBV yield samples, 65 (1/6,012 blood units) were reported as true OBI when retested by NATHBV and dHBV (presence of HBV DNA, dHBV positive with undetectable HBsAg), anti-HBc 98% positive with or without anti-HBs and 2% anti-HBC negative. 41 samples were undefined with a variety of discriminated NATHBV results, characterized by an initial presence of HBV DNA followed by the absence of HBV DNA, dHBV negative with undetectable HBsAg and 90% positivity of anti-HBc. These donors were assessed as HBV inconclusive pending follow-up testing, keeping in mind that HBV DNA is often only intermittently detectable in OBI. All implicated units were discarded. The management strategy for the OBI donors is counseling, debarring from further donations and referral to a hepatologist.
Summary/Conclusions: The complexity and intricacy of confirming OBI cases is a serious concern for blood safety and donor management. Cases with initial but unstable positivity in NATHBV assays due to very low viral load need further molecular investigation by real-time PCR and DNA sequencing. For the undefined and inconclusive cases, retesting (HBsAg, NAT HBV and dHBV, anti-HBc and anti-HBs) within one to three months is required. Post-transfusion information procedures are also in place to estimate the risk of HBV infectivity due to OBI.

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A STUDY ON OCCULT HBV INFECTION AMONG IRANIAN HCV-INFECTED BLOOD DONORS

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Background: Hepatitis C virus (HCV) is a member of the family Flaviviridae and is transmitted through contact with the blood of infected persons. Hepatitis B virus (HBV) is a member of the Hepadnavirus family and is transmitted by the parenteral route. Acute infection of HBV in HCV-infected patients showed that delayed HBsAg appearance with a shorter production of HBsAg compared to those with acute HBV alone.
Aims: The aim of this study was to investigate prevalence of occult HBV infection in Iranian HCV-infected blood donors.
Methods: In this study cross-sectional, 120 HCV-infected blood donors that were HBsAg and anti-HIV negative and anti- HCV positive that their results of confirmatory testing were positive selected for this study. Anti-HBc Ab testing was done for all samples. Also, HBV-DNA PCR was performed on all patients.
Results: Out of 120 HCV-infected blood donors, 19(15.8%) samples were anti-HBc Ab positive. none of them was HBV –DNA PCR positive.
Summary/Conclusions: In this study, HBV-DNA in blood donor's serum with hepatitis C and anti-HBc-positive, and HBsAg negative weren't observed. So the probability of false positive anti-HBc results or past infection with hepatitis B may be considered. Therefore, it is necessary to perform anti-HBs antibody testing for differentiation of past infection.

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HEPATITIS B AND C PREVALENCE IN GUINEA-BISSAU – IMPLICATIONS FOR BLOOD TRANSFUSION

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Background: Before donation and transfusion of blood product, donors should be tested for transmittable pathogens such as HIV, hepatitis B virus (HBV), and hepatitis C virus (HCV). In sub-Saharan Africa these viruses are endemic, which makes it more challenging to find suitable donors. Due to limited resources and time consumption performing screening tests, priority should be given to the most prevalent and pathogenic viruses. Childhood HBV vaccination was implemented in Bissau August 2008. There is no published information on the prevalence of HBV and HCV in the general population in the West African country Guinea-Bissau.

Aims: To estimate the prevalence of HIV, HBV and HCV in the adult general population of Guinea-Bissau.

Methods: Inhabitants of 412 houses in Bissau were randomly selected for inclusion in the survey. This corresponded to 10% of the population from three suburbs at the Bandim Health Project demographic surveillance site. The survey was conducted from November 2014 to February 2016. All participants had a questionnaire filled out and a blood sample collected. HIV testing was performed in Guinea-Bissau, after which samples were shipped to Denmark. HBV and HCV serology was conducted in Denmark using a chemiluminescence assay (Architect, Abbott, Illinois, USA).

Results: Of the 3,127 eligible adults, 2,603 (83.2%) individuals participated in the survey and 2,517 (80.5%) had given sufficient plasma to perform hepatitis analyses. The overall HBsAg prevalence was 19.0%. The highest prevalence was found among the 25–34 year old (22.3%) and decreased with age; 20.0% among the 35–44 years old, 16.3% among 45–55 years old and 8.1% among individuals older than 55 years ($P < 0.01$). Men had a higher HBsAg prevalence than women (24.1% vs 15.3%) with a risk ratio of 1.61 after adjusting for age at inclusion ($P < 0.01$). Only 7.9% did not have any positive HBV marker (HBsAg, anti-HBs and anti-HBc combined). The prevalence of combined HBV-positivity was lower among the 15–24 year old individuals (82.7%) than older individuals (all age groups >95% seropositivity).

A total of 1.9% of the study participants were anti-HCV positive with no differences between sexes ($P = 0.84$). The anti-HCV prevalence increased with age; 1.3% among 15–24 year old 1.2% among 25–34 year old, 1.9% among 35–44 year old, 3.3% among 45–54 year old, and 5.4% among participants older than 54 years.

HIV was detected among 153 (6.1%) of the study participants (94 HIV-1, 57 HIV-2, and 2 HIV-1/2 dual-infected). There was no association between the prevalences of HIV and HBV, or HIV and HCV. After testing risk factors (sexual risk behavior, previous blood transfusion, and other demographics) there were no other associations with neither HBV nor HCV infection. If excluding all HBsAg, anti-HCV and HIV positive individuals, only 76.4% of the population remained as potential blood donors.

Summary/Conclusions: In the general population of Guinea-Bissau HBV was highly endemic with more than 90% having a positive serological marker and almost 20% were HBsAg positive. HCV was most prevalent among the elderly population. If testing for HBsAg, anti-HCV and HIV 24.6% of the population would be excluded as blood donors.

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Abstract has been withdrawn.

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EVALUATION OF HBCAB AND HBSAG IN COMPARISON WITH POLYMERASE CHAIN REACTION FOR THE DIAGNOSIS OF HEPATITIS B VIRUS INFECTION

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Background: Hepatitis B is among the leading life threatening infections in the developing countries. Various screening assays with differing sensitivities and specificities are available for HBV blood screening. The most commonly used screening assay for Hepatitis B is based on detection of HBsAg. Some blood centres also screen for HBcAb. Although HBcAb testing has a definite role in improving blood safety, the wastage of the blood units is a serious issue as up to 10% of the blood collections may have to be discarded on the basis of this testing.

Aims: The objective of the study was to assess the performance and diagnostic effectiveness of HBsAg and HBcAb assays in comparison with the PCR (polymerase chain reaction) for screening of HBV infection.

Methods: This single centre prospective study was conducted at the Department of Blood Transfusion Services, Shaheed Zulfiqar Ali Bhutto Medical University, Islamabad from November 2015 – August 2016. A total of 394 samples were randomly selected and the HBsAg and HBcAb tests were conducted on Abbot ARCHITECT i2000SR Chemiluminescence Immunoassay. Positive PCR results/samples were taken as gold standard for the detection of HBV DNA.

Results: Out of the 394 samples, 47.2% (186/394) were reactive for HBsAg and 52.79% (208/394) non-reactive. When tested for the HBcAb assay, 53.2% (210/394) samples were reactive and remaining were non-reactive. All 394 samples were tested for PCR and showed positivity rate of 47.7% (188/394) close to HBsAg assay outcome. The sensitivity of HBsAg assay was 98.9% (95% CI: 94.2–99.9%) while that of HBcAb assay was 89.5% (95% CI: 82.0–94.6%). The specificity of the HBsAg assay was 98% (95% CI: 94.7- 99.9) while for HBcAb, it was 99.5% (95% CI: 83.3–95.0%). The Positive Predictive Values (PPV) were 90.38% (95% CI: 94.2–99.2%) for HBsAg and 89.5% (95% CI: 82.0–94.6%) for HBcAb. The Negative Predictive Values for the HBsAg assay was 99.0% (95% CI: 94.7–99.9%) but 90.35% (95% CI: 83.3–95.0%) for HBcAb assay. The Negative Diagnostic Likelihood Ratio (NDLR) and Positive Diagnostic Likelihood Ratios for HBcAb were 0.12% (95% CI: 0.07%>0.20%) and 9.28 (95% CI: 5.27%>16.33%) respectively while Negative Diagnostic Likelihood Ratio (NDLR) and Positive Diagnostic Likelihood Ratios for HBsAg assay were observed as 0.12 (95% CI: 0.07%>0.20%) and 10.2 (95% CI: 14.63%>723.77%) respectively. The Disease Prevalence for HBsAg and HBcAb assays were 47.7% (95% CI: 40.63%> 54.92%) and 47.9% respectively (95% CI: 41.17%>54.78%). The test agreement, i.e. Kappa Agreement between the HBsAg and HBcAb assays was high both for HBsAg detection ($\kappa = 0.96$; 95% CI: 0.93%> 0.99) and for HBcAb detection ($\kappa = 0.88$; 95% CI: 0.85%> 0.93%). The false positive rate of HBsAg was observed as 0.5% and false for HBcAb was 5.5% as compare with the gold standard (PCR).

Summary/Conclusions: PCR is an expensive approach for a developing country like Pakistan. Therefore, only HBsAg screening should be considered as a best alternative and give satisfactory results during the window time of infection.

P-329

PREVALENCE OF HEPATITIS B AND HEPATITIS C VIRUSES IN BLOOD DONORS AT ZLATIBOR REGION (9 YEARS EXPERIENCE)

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Background: Risk of transfusion transmissible infections depends on prevalence of infectious agents in the general population (particularly in blood donors population) and the presence or absence of immunity of the recipients to the infection. Hepatitis is known as one of the most severe complications of transfusions hemoproducts.

Aims: This study was aimed to determine the degree of hepatitis B and C prevalence in blood donors throughout 9 years time interval, analysis according to the number of blood donation, sex and age of blood donors.
Methods: Between January 2008 and December 2016, 69,989 blood donors were tested for HBV and HCV using immunochemical assays (enzyme immunoassays, chemiluminescent microparticle immunoassay). Initially reactive samples were retested two times. The reactive cases were confirmed Western blot method as per the national algorithm.
Results: Of the totally tested 69 989 blood donors preliminary reactive samples were 92 HBsAg (0.13%) and 118 HCV (0.17%). Reactive blood donors were detected 50 HBsAg (0.071%) and 13 HCV (0.018%). 44 HBsAg (0.063%) and 13 HCV (0.018%) reactive blood donors were male donors.47 HBsAg (0.067%) and 9 HCV (0.013%) was first donated blood. HBsAg prevalence was highest among blood donors in the age group 40–49 (22), and 50–65 (15) and HCV 30–39 (4), 40–49 (3) and 50–65 (3).
Summary/Conclusions: Prevalence of HBsAg and HCV in the Zlatibor region extremely low compared with other regions. The largest number of reactive blood donors was at providers that make the first time blood and older than 40 years. To further reduce the prevalence of HBsAg vaccination is important newborns introduced by law.

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Abstract has been withdrawn.

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PERFORMANCE OF A NEW AUTOMATED ASSAY FOR HEPATITIS B SURFACE ANTIGEN AND HEPATITIS B SURFACE ANTIGEN CONFIRMATORY ON THE ALINITY S SYSTEM

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Background: Despite the development of sensitive NAT methods, blood transfusion in many parts of the world relies on serologic screening for Hepatitis B surface antigen (HBsAg) to prevent transfusion transmitted HBV infection. Sensitive HBsAg assays must be capable of coping with a wide range of mutants while exhibiting an uncompromised specificity. In addition, continued pressures on laboratory operations demand that assays perform on platforms capable of increased walk away time and enhanced automation in areas of reagent management, retest options, and commodity/waste management.
Aims: To evaluate the overall performance of a new automated prototype chemiluminescence immunoassay for the detection and confirmation of HBsAg on an automated next generation platform, Alinity s.
Methods: The performance of the automated prototype immunoassay for the detection and confirmation of HBsAg was evaluated on a next generation automated platform, Abbott Alinity s. Precision was assessed over 20 days. Sensitivity was evaluated using 412 known positive samples, 30 commercially available seroconversion panels, the WHO standard, 23 HBsAg mutants, and 94 HBsAg genotyped specimens (A through H). Specificity was evaluated on random blood donors and plasmapheresis donors.
Results: Precision was less than 8% CV for positive samples over 20 days. The blood donor specificity was 99.97% (6,073/6,075). Sensitivity was 100% for 412 presumed positive samples. Sensitivity was 100% for all genotypes. 100% of the mutants were detected vs 83% for the comparator. Seroconversion detection was as good as the comparator assay with 157 reactive samples detected with the Alinity s assay and 154 reactive samples detected by the comparator assay. Analytical sensitivity ranged from 0.015 to 0.016 IU/ml. The Alinity s HBsAg Confirmatory Assay confirmed all known positive HBsAg specimens, including 3 HBsAg mutant samples that were not confirmed by the comparator HBsAg Confirmatory Assay.
Summary/Conclusions: The new automated prototype Alinity s HBsAg assay provided precision, specificity, and sensitivity comparable to the current on-market comparator assay. However, the HBsAg assay demonstrated a gain in sensitivity over the comparator assay through the detection and confirmation of a wider range of mutants.

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COMPARATIVE STUDY ON THE CONFIRMATION TESTS FOR HEPATITIS B VIRUS INFECTION IN DONATED BLOOD

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Background: Determination of hepatitis B virus (HBV) infection in donated blood is considered as mandatory regulation. Currently there are two standard tests, which are serological (detect HBsAg) and molecular (detect HBV DNA) methodology. In the past at National blood centre, all CMIA initial reactive samples were retested by duplicate test and the later study for compared method of the duplicate test, neutralization and mini pool of 6-NAT. Result was shown that samples exhibited false reactive duplicate test, given S/CO value <1,000, 80% of samples of initial reactive CMIA with S/CO ≥1,000 showed reactive results by neutralization and had to be more diluted due to an exceeded amount of HBsAg. Since January 7th, 2016, the NAT has changed from mini pool of six samples to be an individual sample tested instead. Our research team would like to investigate and compare between various methods used in routine laboratory practice.
Aims: To validate accuracy and reliability for confirm HBV infection in donated blood due to the newly modified methodology.
Methods: 1. The samples were from the division of routine screening, during August to October 2016. 2. Samples initially reactive CMIA for HBsAg with the S/CO <1,000 were retested twice (repeated duplicate testing), followed by detecting HBsAg neutralization and NAT. 3. The result of each sample was recorded in Excel program for further analysis. 4. Data analysis and classification were performed by Excel program and 2 × 2 table.
Results: A total samples were 169,969, yielding a preliminary test of 595 reactive samples. Of these, 253 samples (0.15%) exhibited the value of S/CO <1,000, whereas. This study showed that, of 253 specimens the repeated duplicate test yielded 128 reactive samples. Within this reactive sample group, 48 and 41 samples showed reactive by Neutralization and NAT, respectively. On the other hand, within the negative sample group, one sample showed reactive result for neutralization, and two samples for NAT. Comparing results from both neutralization and NAT methods, 41 samples were concordantly reactive, 202 samples were negative, and 10 showed inconsistent test results.
Summary/Conclusions: Using repeated duplicate testing, 50% of samples were shown to be positive result, while neutralization and NAT yielded positive results for 19% and 17%, respectively. Within positive samples by repeated duplicate testing, they were also tested positive by neutralization and NAT as 38% and 32%, respectively. The repeat duplicate testing was shown to be unreliable; especially in samples that S/CO lower than 1,000, resulting in wasteful expenditure and discarding these donated bloods. Blood donors have to stop donating blood more than using neutralization at 62% and NAT at 68%. Both neutralization and NAT methods exhibited mostly concordant results, 16% of samples were positive, 80% were negative, but only 4% of samples showed discordant result. There were 3% of specimens showed positive by neutralization but negative by NAT, 0.8% of samples showed negative by neutralization but positive by NAT. This study demonstrated that determination of HBV infection in donated blood was necessary to consider results from both neutralization and NAT methods, provide the highest quality of safety blood for all patients.

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PREVALENCE OF HEPATITIS B CORE ANTIBODY ONLY POSITIVE AMONG HEPATITIS B SURFACE ANTIGEN NEGATIVE BLOOD DONORS IN MANDALAY GENERAL HOSPITAL, MYANMAR

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Background: Blood transfusion is essential and saves millions of lives. There are about 32,000 blood donations per year in Blood Bank, Mandalay General Hospital, Myanmar. The risk of transfusion transmitted infections has been reduced markedly, however a zero risk blood supply remains a popular goal. Hepatitis B infection is one of the most frequent post transfusion infections although sensitive screening assays for detection of HBsAg are available. Screening of HBsAg alone is not sufficient to eliminate transfusion transmitted hepatitis B infection due to the pre-seroconversion window period, infection with immunovariant viruses, and with occult carriage of HBV. Implementation of highly sensitive HBV DNA screening is used in many countries but it cannot be used in developing countries like Myanmar. This

study could provide useful information for implementation of new strategies for transfusion transmitted hepatitis B infection.
Aims: The aim is to study prevalence of AntiHBc only positive among HBs Ag negative blood donors and to evaluate the infectivity of blood components from such donors in Mandalay General Hospital, Myanmar.
Methods: Blood donors who were HBsAg negative by chemiluminescence immunoassay (Cobas e411) and did not receive HBV vaccination were collected after informed consent. Testing of AntiHBc was done with Cobase411. Blood sample positive for AntiHBc were tested for AntiHBs with Cobase411.
Results: Among 176 HBs Ag negative first time non vaccinated blood donors, 71 donors (40.34%) were positive for AntiHBc. The commonest age group was 18–28 year group (137 donors, 77.84%), followed by 29–38 year group (33 donors, 18.75%). Male to female ratio was 2.7:1. The age group of 18–28 year was the commonest with 51 AntiHBc positive donors (37.23%). Among 71 AntiHBc positive donors, 13 donors (18.31%) were AntiHBs negative (<10mIU/ml) which meant no immunity for HBV. Sixteen donors (22.53%) had AntiHBs <100mIU/ml that meant although there were AntiHBs positive, they did not have protective level for HBV infection at that moment but they may arise high titre with time. Forty two donors (59.15%) had AntiHBs >100mIU/ml. Therefore those 42 donors had immunity for HBV and their blood donations can be used safely for clinical purpose. In 176 HBs Ag negative blood donors, 13 donors were AntiHBc only positive (7.4%). As a result of this study, 7.4% hence might be infectious.
Summary/Conclusions: The prevalence of AntiHBc only positive in HBsAg negative blood donors is 7.4%. If those blood were used, they might give transfusion transmitted hepatitis B infection. According to this study, testing of HBsAg alone is not sufficient to reduce the risk of transfusion transmitted HBV infection. For those donors with HBs Ab negative, AntiHBc detection and HBV DNA testing should be done for exclusion of occult B infection. Detection of HBs Ab titre is useful in AntiHBc positive donors unless NAT is available. Simultaneous screening of HBs Ag and HBs Ab by chemiluminescence immunoassay is time saving and effective. If those donors have HBs Ab titre of protective level, the blood can be used safely. This result will provide useful information for implementation of new strategies for transfusion transmitted hepatitis B infection in blood transfusion in high prevalence developing countries. The present study had some limitations that need consideration. This study was a uni-centre study with a small sample size. Therefore, the results did not necessarily represent for all populations in other developing countries.

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HEPATITIS B CORE ANTIBODY REACTIVITY AMONG VOLUNTARY NON-REMUNERATED BLOOD DONORS VERSUS REPLACEMENT BLOOD DONORS IN PAKISTAN

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Background: There is a moderate prevalence of Hepatitis B in Pakistan. Occult Hepatitis B Virus infection might result in transmission of Hepatitis B to the patient, if blood is only tested for Hepatitis B surface antigen. Implementing anti Hepatitis B core (anti-HBc) testing on blood donors decreases the risk of Hepatitis B virus transmission. Globally, Voluntary Non-Remunerated Blood Donors (VNRBD) are known to have a lower prevalence of transfusion transmitted infections.
Aims: To compare the prevalence of anti-HBc reactivity amongst non-remunerated replacement and VNRBD. In addition, to identify the prevalence of anti-HBc in VNRBD of different sectors of Karachi, Pakistan.
Methods: In the period of 37 months (Dec 2013 – Dec 2016), 45,543 blood samples were tested for anti-HBc, where 38,475 (84.5%) were VNRBD and 7,068 (15.5%) were non-remunerated replacement donors. All testing was performed using the Chemiluminescent Microparticle Immunoassay (CMIA) method on Abbott Architect *i*2000SR.
Results: In VNRBD, 3,962 out of 38,475 (10.3%) and in non-remunerated replacement donors 1,160 out of 7,068 (16.4%) ($P < 0.001$) were reactive for anti-HBc. Amongst the VNRBD, anti-HBc reactivity was 15.3% in factories (15,752 donors drawn), 13.4%, in religious seminaries (Madrassas) (2,769 donors drawn), 10.2% in miscellaneous group (1,990 donors drawn), 9.0% at sites of worship (1,656 donors drawn), 6.5% in corporate offices (6,151 donors drawn) and 3.5% in colleges/universities (8,922 donors drawn).
The reactivity was significantly higher ($P < 0.001$) for donors belonging to low socioeconomic areas (14.5%) as compared to the affluent areas of Karachi (5%). Furthermore, donors who are below 30 years of age were found to have significantly ($P < 0.001$) lesser reactivity (8.8%) compared to those above 30 years of age (13.8%).
Summary/Conclusions: In Karachi Pakistan, prevalence of anti-HBc was significantly higher in non-remunerated replacement donors compared to VNRBD. Of the latter, the highest reactivity was noted in factories and religious seminaries (madrassas). Whereas, the lowest reactivity was noted in higher educational institutions followed by the corporate sector. Donors above 30 years of age, comparatively had a higher prevalence. Moreover, donors residing in affluent socioeconomic areas had a lower prevalence of anti-HBc.
The higher prevalence of anti-HBc amongst non-remunerated replacement donors in Pakistan proves that blood supply from VNRBD is the safest, specifically drawn from young population of higher educational institutions.

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Abstract has been withdrawn.

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PERFORMANCE OF NEW AUTOMATED IMMUNOASSAY ASSAY FOR ANTI-HBC ON THE ALINITY S SYSTEM

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Background: In countries with a low prevalence of Hepatitis B, blood donations are commonly screened to detect the presence of antibodies to hepatitis B core antigen (Anti-HBc) alongside HBsAg and HBV NAT to detect donors with occult Hepatitis B infections (OBI). Blood centers require anti-HBc assays with high specificity and sensitivity to prevent unnecessary donor deferrals while maintaining a safe blood supply. In addition, continued pressures on laboratory operations demand that assays perform on platforms capable of increased walk away time and enhanced automation in areas of reagent management, retest options, and commodity/waste management. In the response for the need for such screening assays, we have evaluated an improved automated assay for the detection of anti-HBc.
Aims: To evaluate the overall performance of a new chemiluminescence immunoassay for the detection of anti-HBc antibodies on a next generation automated platform, Alinity s.
Methods: The performance of the new chemiluminescence immunoassay for the detection of anti-HBc was evaluated on the Abbott Alinity s platform. Precision was assessed over 20 days evaluating positive samples. Specificity was evaluated on samples obtained from random blood donors. Sensitivity was evaluated using specimens characterized as anti-HBc positive using serologic methods. Analytical sensitivity was assessed using the WHO 1st International standard. Seroconversion sensitivity was evaluated with 10 commercial seroconversion panels.
Results: Precision was less than 6% CV for positive samples over 20 days. The blood donor specificity was 99.90% (5,098/5,103). Sensitivity was 100% for 400 samples presumed to be anti-HBc positive. Analytical sensitivity results on the Alinity s Anti-HBc assay ranged from 0.57 to 0.62 IU/ml. Seroconversion detection was as good as the comparator assay with 136 reactive samples detected with the Alinity s assay and 134 reactive samples detected by the comparator assay.
Summary/Conclusions: These results indicate that the new automated Alinity s Anti-HBc assay provided acceptable performance in specificity, sensitivity and precision versus the comparator assay.

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RESULTS OF ROUTINE SCREENING ALL DONATIONS FOR HEPATITIS B CORE ANTIGEN ANTIBODIES

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Background: Viral hepatitis B widely spread. So does its latent forms. Hepatitis B virus (HBV) core antigen antibodies (a-HBc) screening reveals the most cases of occult HBV infection (OBI). HBV DNA testing of blood samples from the donors with OBI and low viremia may be false negative. Transfusion of occult infected blood may lead to acute hepatitis B especially in immunocompromised recipients.
Aims: Assess the impact of the a-HBc blood testing implementation for viral safety increasing.
Methods: Since March 2014, National Research Center for Hematology's blood testing protocol includes a-HBc for every single donation. In the period from March

2014 to April 2016 26 113 donor samples was screened by Bio-Rad Monolisa a-HBc Plus and Abbott Anti-HBc II. Seronegative samples were tested in PCR by Cobas TaqScreen MPX Test, version 2.0 in pools of six. Blood recipients (patients with hematological malignancies) were monitored for HBV markers (HBsAg, HBV DNA).
Results: A-HBc was found in 621 from 26 113 samples (2.4%). In 15 samples were also found HBsAg, in 4 – *Treponema pallidum* antibodies, in 3 – hepatitis C virus antibodies and in 1 – human immunodeficiency virus antibodies. Thus among all 621 a-HBc positive samples, 598 were positive only for anti-HBc. Among seronegative samples, tested by PCR for HBV DNA, HCV RNA and HIV RNA, 1 sample was positive for HBV DNA. Donor, in which blood was found HBV DNA, subsequently showed acute viral hepatitis symptoms. Thus initial HBV infection in donor was detected. All blood collected from these donations were discarded.
In the period from August 2013 to October 2014 (15 months follow-up) five blood recipients showed laboratory signs of primary HBV infection. The results of epidemiological investigation did not exclude transfusion route of infection in one case. Since October 2014 (6 months after the introduction of routine a-HBc screening) at the time of January 1, 2017 (27 months follow-up), primary HBV infection detected only in one patient. The epidemiological investigation of this case did not confirmed transmission of HBV through transfusion route.
Summary/Conclusions: Routine anti-HBc screening provides extra detection of occult HBV infected donors and in complex with DNA HBV testing completely prevents possible transfusion of infected blood. Efficiency of this protocol of laboratory examination of donor's blood for enhancing of blood transfusions viral safety is confirmed by absence of new cases of HBV infection in recipients.

Hepatitis C (HCV)

P-338

SELECTIVE AND SENSITIVE CHEMILUMINESCENCE FOR DETECTION OF HCV ANTIGEN- SPECIFIC ANTIBODIES IN EGYPTIAN NATIONAL BLOOD TRANSFUSION CENTER (ENBTC)

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Background: The diagnosis and monitoring of hepatitis C virus (HCV) infection are based on two types of tests: a serological test that detects HCV antigen-specific antibodies and assays that detect viral RNA or HCV core antigens.
The presence of anti-HCV antibodies does not always indicate the existence of a current infection; instead, it may represent a false positive result as may occur due to interfering factors such as high gamma globulin levels, nephritic syndrome, liver diseases, autoimmune diseases, viral or parasitic infections or pregnancy in women.
In the Egyptian National Blood Transfusion Centre, the detection of anti-HCV antibodies is routinely performed using two different chemiluminescence immunoassay methods.
Aims: To compare between the specificity of two CLIA assays in the results of screening for Anti HCV antibodies in ENBTC.
Methods: 100 samples were simultaneously tested for HCV antibodies on both systems under routine laboratory conditions. Samples that were initially reactive on either system were repeated in duplicate using same sample and same assay in accordance with the national screening algorithm followed. Samples that were repeatedly reactive on the Sensitive device and non-reactive on the selective device were further tested using second generation (Immunoblot Assay).
Results: - 22 samples were repeatedly reactive using the sensitive device method, whereas 78 were non-reactive.
- 92 samples were non-reactive using the selective device method and eight samples were repeatedly reactive.
- The difference in repeatedly reactive samples on both systems was14 samples.
- These 14 samples were further tested using second-generation Recombinant Immunoblot Assay. The results of these samples were non-reactive indicating false positivity by screening on the sensitive device.
Summary/Conclusions: According to the results obtained, one of CLIA assay appears to be less sensitive than the other. However, the confirmatory test used (the immunoblot assay) proved the Anti-HCV assay on the selective device to be more specific for the detection of anti-HCV reactive blood units.
The results adds to the ongoing struggle in developing countries, such as Egypt, between the necessity of having a Nucleic Acid Test in addition to the immunoassays routinely used for screening, and the difficulty of having sustainable funds.
blood units in countries where shortage of blood is already there together with high prevalence of TTIs.
Within the ENBTC, further research work should be conducted on larger scale of samples to determine the exact wastage of false reactive blood units that could be saved using additional testing to confirm the reactivity.

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PERFORMANCE OF A NEW AUTOMATED ASSAY FOR ANTI-HCV FOR THE ALINITY S SYSTEM

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Background: Serological screening for antibodies to Hepatitis C virus (HCV) often in conjunction with nucleic acid testing (NAT) is used worldwide to prevent transfusion transmitted HCV infections. While NAT provides improved sensitivity and detection of HCV in the pre-seroconversion window, serological testing provides continued detection of HCV in infected individuals and individuals with resolved infections with no detectable HCV RNA. Blood and plasma centers require very high throughput anti-HCV assays with high specificity and sensitivity to prevent unnecessary donor deferrals while maintaining the safety of the blood and plasma supply. In addition, continued pressures on laboratory operations demand that assays perform on platforms capable of increased walk away time and enhanced automation in areas of reagent management, retest options, and commodity/waste management.
Aims: The performance of a new automated chemiluminescence immunoassay for the detection of antibodies to HCV was evaluated on the Alinity s system.
Methods: Precision was assessed over 20 days evaluating positive samples. Sensitivity was evaluated on 401 preselected positive samples and 30 seroconversion panels. Specificity was evaluated on samples obtained from 6,189 blood donors from the US and Europe and 200 diagnostic samples obtained from the US. Sensitivity and specificity samples were split across three reagent lots during testing. Confirmation of repeatedly reactive samples was performed using an algorithm consisting of the INNO-LIA™ HCV Score and NAT/HCV Discriminatory NAT assays.
Results: Precision was less than 7.0% CV for positive samples over 20 days. Overall clinical sensitivity was 100% on 401 preselected anti-HCV positive samples. Seroconversion sensitivity was better than the comparator as evidenced by the Alinity s Anti-HCV assay identifying five more bleeds than the comparator assay. The specificity was 99.98% (6,187/6,188) on blood donor population and 100.00% (200/200) on a diagnostic population.
Summary/Conclusions: These results indicate that the new automated Alinity s Anti-HCV assay provided very good performance in precision, specificity and sensitivity.

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Abstract has been withdrawn.

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SEROPREVALENCE OF HEPATITIS C IN VOLUNTARY NON-REMUNERATED BLOOD DONORS VERSUS REPLACEMENT BLOOD DONORS IN PAKISTAN

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Background: Pakistan has a moderate prevalence (1.5–3.5%) of Hepatitis C. Unsafe blood transfusion being one of the major causes. The major source of blood in Pakistan is replacement donors provided by the patients' family and friends. Voluntary Non-Remunerated Blood Donors (VNRBD) are known to have a lower prevalence of transfusion transmitted infections.
Aims: To investigate the prevalence of Hepatitis C in replacement and VNRBD in different sectors of the cosmopolitan city of Karachi.
Methods: Over a period of 37 months (Dec 2013 to Dec 2016), 40,411 healthy donors were drawn, of which 34,496 (85%) were VNRBD and 5,915 (15%) were non-remunerated replacement donors. All donors were screened for Hepatitis C antibodies

(Anti-HCV) using Chemiluminescent Microparticle Immunoassay (CMIA) method on Abbott Architect *i*2000SR.
Results: Amongst VNRBD, 477 (1.38%) out of 34,496 tested reactive for Anti HCV which was significantly lower ($P < 0.001$) compared to 125 (2.11%) out of 5,915 in non-remunerated replacement donors.
Reactivity of Anti-HCV in all donors above age 30 (1.8%) was statistically significant ($P < 0.001$) compared to those below 30 years of age (1.2%).
Prevalence of Anti-HCV in donors residing in affluent areas was 0.66% compared to 1.71% in donors residing in low socioeconomic areas ($P < 0.001$).
Amongst VNRBD, factories had the highest reactivity 1.95% (13,865 donors tested) followed by religious seminaries (madrassas) 1.71% (2,519 donors tested), miscellaneous group 1.34% (1,785 donors tested), corporate offices 1.02% (5,896 donors tested), sites of worship 0.65% (1,543 donors tested) and colleges/universities 0.57% (9,100 donors tested).
Summary/Conclusions: These results give evidence to the safety of VNRBD compared to non-remunerated replacement donors in Karachi, Pakistan. Amongst VNRBD, highest reactivity was noted in factories and religious seminaries, where majority of the donors belong to low socioeconomic groups. Furthermore, lowest reactivity was noted in donors below 30 years of age in higher educational institutions and donors residing in affluent areas.

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THE USEFULNESS OF ANTI-HCV SIGNAL TO CUT-OFF RATIO IN PREDICTING VIREMIA IN ANTI-HCV IN PATIENTS WITH HEPATITIS C VIRUS INFECTION

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Background: Hepatitis C Virus (HCV) infection is diagnosed by antibody and RNA based methods. Patients with anti-HCV sample rate/cutoff rate (S/CO) ratios > 1 are reported as anti-HCV positive. RNA based methods are introduced to confirm positivity in seropositive samples.
Aims: The current study aimed to assess relationship between S/CO rates and HCV-RNA levels in the laboratory to identify HCV viremia in patients with a positive anti-HCV.
Methods: All serum samples were assayed for anti-HCV by ELISA method. A total of 265 anti-HCV positive patients were tested for HCV-RNA testing by quantitative method using Artus HCV RG Real-time Polymerase Chain Reaction (RT- PCR) kit. Statistical analysis was done by SPSS version 16.
Results: Of the 265 patients with HCV infection, 204 (77%) were male and the mean age was 43.53 ± 13.17 years, ranging 1–81 years. No correlation was found between S/CO ratios and HCV-RNA levels. There was significant difference in S/CO ratio between viremic and non-viremic subjects. The sensitivity, specificity, negative predictive value, and positive predictive value were 100%, 81.4%, 100%, and 77.2%, respectively in the S/CO ratio of 2.7.
Summary/Conclusions: The present study indicated that anti-HCV S/Co ratio is useful to predict non-viremic patients. A cut-off value of 2.7 can determine the usefulness of HCV-RNA testing. Patients with S/CO <2.7 are not viremic; therefore, HCV-RNA testing is not recommended. It is suggested that laboratories report S/CO ratio along with anti-HCV results to manage HCV infection better, especially in countries that quantitative HCV testing is expensive or not available.

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Abstract has been withdrawn.

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SEROPREVALENCE OF INFECTIOUS MARKERS AMONG BLOOD DONORS IN PAKISTAN-TREND OVER A DECADE

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Background: Blood transfusion is a lifesaving therapeutic intervention but is not without risks. One of the major risks of blood transfusion includes transmission of infections. Although, the incidence rates across the world have declined due to the awareness but developing countries are still in the state of war against these killer infections.
Aims: The aim of this study was to determine the seroprevalence and temporal trend of infectious disease markers in blood donors in Pakistan over the last decade.
Methods: This retrospective descriptive study was conducted at the Blood Bank of The Aga Khan University Hospital from 1st January 2005 to 30th September 2016. Infectious disease tests were performed on blood samples of all donors (replacement and voluntary). Every donation (100%) was screened for anti-HIV-1/2 [Third generation automated chemiluminescence immunoassay (CLIA)], hepatitis C virus antibody (anti-HCV by CLIA), hepatitis B surface antigen (HBsAg by CLIA), venereal disease research laboratory test (VDRL) and Immunochromatography for malaria (ICT malaria). All reactive tests were repeated for concordance. Data for positive screening result was collected from blood bank information system. Statistical package for social sciences 19 was used for data entry and analysis. Prevalence and trend of HbsAg, anti HCV, anti- HIV, VDRL and ICT malaria was calculated in blood donors.
Results: Total of 262,819 blood donors donated blood over last ten years. Ninety-five percent of the donors (n = 249,678) were replacement blood donors, the rest (n = 13,141; 5%) being voluntary donors. Total number of sero reactive donors was 9,669 (3.7%). Prevalence of infectious markers was as follows: anti-HCV [n = 4,688 (1.8%)], HBsAg [n = 3,482 (1.3%)], anti-HIV [n = 145 (0.05%)], VDRL [n = 1,252 (0.5%)] and ICT malaria [n = 102 (0.04%)]. The seropositivity for anti-HCV fluctuated between 1.7% and 2.1% during last ten years. HBsAg remained static at a rate of 1.3%. VDRL prevalence varied from 0.3% to 0.6%. Anti-HIV prevalence ranged from 0.02% to 0.07%. ICT malaria showed gradual reduction from 0.01% to 0.004%.
Summary/Conclusions: The bulk of donors in this study included replacement donors (95%). Anti-HCV was the most prevalent infectious disease marker (1.8%) detected in our blood donors followed by HBsAg (1.3%). There was no significant change in the trend for all infectious disease markers except ICT malaria which has gradually declined over the decade.
The trend shows that both Hepatitis C and B continue to pose a risk for transfusion in our population. The risk of transmission of these infectious agents could be reduced with implementation of strict donor criteria and Nucleic acid amplification technique (NAT) across the country.

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FOLLOW-UP OF ONE VOLUNTARY BLOOD DONOR FOR NUCLEIC ACID TEST HCV RNA REACTIVE

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Background: Nucleic acid test (NAT) as a high sensitivity assay has been introduced to reduce the residual risk of transmission of HCV by transfusion of blood components. It can detect the contaminated blood which is caused by the "window period" (WP) infection, immuno silent infection, mutation of the virus and so on. This study was planned for follow-up of hepatitis C virus (HCV) NAT reactive and antibody non-reactive blood donor.
Aims: To observe the antibody seroconversion of the HCV RNA+/Ab- blood donor and confirmed the" window period "infection of HCV.
Methods: Roche Cobas's201 and KE HUA NAT system were performed to detect jointly HBV/HCV//HIV in the voluntary blood donors' negative samples which were tested by ELISA reagents for HBsAg/anti-HCV/anti-HIV1/2. Automated detection algorithm of samples mixed pools was performed, and then each donor sample of reactive pools was separately detected. Suspected WP infection were follow-up to monitor the development of the immune response.
Results: In the study, 125 NAT reactive samples were found in 247,936 seronegative samples detection (the positive detection rate was 1/1983). One HCV WP infection was confirmed by two NAT systems and the HCV antibody was completely converted to a reactive level by the follow-up of the donor for six weeks. And the ALT levels of the donor tended to achieve rapid enhancement for five weeks and reached in decline in the sixth week. The seventh week results of blood donor showed: HCV viral load: 1.22 $x10^3$ IU/ml, anti-HCV s/co value: 11.4, ALT: 164 U/L. Besides that, the blood donor who had presented five voluntary blood donations since 2009 and had no history of blood transfusion was probably transmitted by his wife. The HCV RNA+ results of his wife was also confirmed in the author's laboratory.
Summary/Conclusions: NAT was used for blood screening, which could help to shorten the "window period" of HCV detection and effectively block the case of transfusion-transmitted disease. The novel assay may be useful for early detection of HCV infection and popularized in the future.

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SOLUBLE HUMAN LEUKOCYTE ANTIGEN-G LEVELS IN HCV PATIENTS

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Background: Human leukocyte antigen (HLA)-G, a nonclassical HLA class I molecule, is known to have tolerogenic properties. There are growing data on the association between soluble HLA-G and viral infections including HCV. Besides, studies on the correlation of the levels of sHLA-G and graft acceptance are available.
Aims: We investigated the expression of sHLA-G levels both in blood donors and in patients on waiting list for kidney transplantation with and without anti-HCV.
Methods: Sera of 23 blood donors with anti-HCV (n = 13) and without anti-HCV (n = 10) were analyzed. Among 67 sera of patients in waiting list for kidney transplantation, n = 43 were with anti-HCV and n = 24 without anti-HCV. All serum samples were tested for anti-HCV by using chemiluminescent immunoassays (CMIA) (ARCHITECT, Abbott Diagnostics, Wiesbaden, Germany) followed by confirmatory testing for HCV (Immunoblot assays, INNO-LIA, Innogenetics, Ghent, Belgium). The serum levels of sHLA-G were analyzed by Enzyme-Linked Immunosorbent Assay (ELISA, BioVendor, Czech Republic).
Results: Preliminary data showed that sHLA-G levels of patient sera were significantly higher compared to blood donors (P = 0.006). Moreover, P for trend of sHLA-G in HCV negative (blood donors and patients) was significant (P = 0.004) while P for trend of sHLA-G in HCV positive (blood donors and patients) was not significant (P = 0.414).
Summary/Conclusions: We found high levels of sHLA-G in waiting list patients compared to blood donors. This confirmed the key tolerogenic role of this molecule. No direct correlation between sHLA-G and the presence of anti-HCV was observed in our groups. The role of sHLA-G in hepatitis remains to be elucidated.

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BEHAVIORAL PROFILE OF PERSONS WITH CHRONIC HEPATITIS C INFECTION IN VOJVODINA

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Background: Chronic hepatitis C virus (HCV) infection is a worldwide public health problem. An understanding of how a person was infected is important for preventing HCV infection. Blood safety depends on the recruitment and retention of blood donors who are at low risk of HCV infection. There are limited data available regarding the behavioral profile of blood donors with HCV infection. Recognizing the relationship between the profiles of HCV infected patients and blood donors may lead to improving blood safety.
Aims: To investigate socio-demographic and epidemiological characteristics in adult patients with chronic HCV infection.
Methods: This study was a retrospective, descriptive study, which included patients with chronic HCV infection who were treated at the Clinic for Infectious Diseases of the Clinical Center of Vojvodina. Patient data were collected using standardized epidemiological questionnaires during the patients' first visit to the clinic. Modes of transmission of HCV infection were classified into 11 categories.
Results: The studied sample consisted of 292 patients with chronic HCV infection, 190 male (65%) and 102 female (35%). These patients had an average age of 38.5 years. The average age of men was 37.8, while women averaged 39.9. Multiple risk factors for acquiring HCV have identified: dialysis 4.5%, intravenous drug use 52.1%, surgery 6.8%, tattoos and piercings 19.9%, blood derivatives 3.8% (patients with hemophilia), receipt of infected blood transfusion 0.3%, professional exposure 0.3%, unknowns mode of infection 12.3%. Three categories (household contact, perinatal, sexual exposure) showed statistical irrelevance and not presented in this study. Steatosis was presented in 18% of patients.
Summary/Conclusions: The data analyzed show that the gender, age and routes of transmission of infection in patients with chronic HCV infection are similar to blood donors date. The majority of HCV infected voluntary blood donors in Vojvodina were male first-time donors (85%). The prevalence of HCV was significantly higher among the age group older than 40. In conclusion, HCV infection appeared to be lower among blood donors than that in the general population. This is largely due to the effective system to select donors and screen donated blood.

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PREVALENCE OF TRANSFUSION-TRANSMITTED INFECTIONS IN TRANSFUSION-DEPENDENT THALASSEMIA PATIENTS AND RELATION TO THE NUMBER OF TRANSFUSED BLOOD: A MULTICENTER STUDY

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Background: Patients receiving chronic transfusion therapy are at particular risk of acquiring viral infections from blood products, especially hepatitis B and C viruses, cytomegalovirus (CMV) and human immunodeficiency virus (HIV), followed by HTLV I-II, human parvovirus B19, and TTV. Transfusion transmitted infections are the second commonest cause of death in thalassemia major. In Turkey, according to the latest official record, a total of 4,500 patients are currently registered with homozygous b thalassemia or other hemoglobinopaties. The patients with thalassemia major constitute the largest group of patients who need regular transfusion program throughout the life in Turkey.
Aims: This study is designated to reveal the seropositivity status of thalassemia major for blood-borne infections and prevalence of transfusion-transmitted infections (TTIs) and relation to the number of blood units transfused. We also aimed to investigate the increasing risk of infections with transfused blood in course of years. The conventional treatment for patients suffering from beta thalassemia is regular blood transfusion and chelation therapy. The patients were transfused by ABO – Rh (D) matched random fresh (donation date is less than a week) red cell concentrates for maintaining pretransfusional hemoglobin levels over 9–10 g/dl. The patients have been vaccinated against HBV and HAV when first diagnosed as thalassemia major. The patients who need regular blood transfusions have been followed up regarding transfusion transmitted viruses including HBV, HCV and HIV, 6 monthly.
Methods: A questionnaire inquiring the demographics and medical history of the patients as well as serological status for hepatitis B, C and HIV, was sent to 12 Thalassemia Centers. The data of 999 thalassemia major patients on regular transfusion program was collected and analyzed.
Results: A total of 999 patients were included in this study. The patients were aged between 1 and 53 years (median 16 years). Total 25 HBsAg (+), 126 anti-HCV (+) and 2 anti-HIV (+) patient were found in 999 patients. Although there is a considerable difference in the frequency of seropositivity between the thalassemia centers, the overall HBsAg and anti-HCV positivity were found 2.9% and 13.4% respectively. The confirmed Anti-HIV positivity was appeared 0.2% in the study group and consistent with the low frequency of the disease among blood donors in Turkey. The % seropositivity within the patients in each particular age groups were as follows: % in 0–5 years, 7.8% in 6–10 years, 18.7% in 11–15 years, 21.9% in 16–20 years and 51.7% in over 21 years.
Summary/Conclusions: Study showed that, 68.16% of all cases positive for anti-HCV were >11 years of age. Seropositivity was 12.65% in 11–15 age group, 13.96% in 16–20 and 24.03% in 21–25 age group for HCV. All the cases of positive anti-HCV had received more than 100 transfusions. Probability of developing anti-HCV is increasing with age, due to cumulative increase in the transfusions, despite questioning the donor, screening the donated blood and contingent transfusion decisions. Proposal for future could be more stringent donor-screening strategies; the promotion of voluntary blood donation; and the implementation of newer technologies including pathogen inactivation technologies.

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HEPATITIS C VIRAL INFECTION PREVALENCE IN MULTI-TRANSFUSED THALASSEMIC PATIENTS IN MYANMAR: PAST, PRESENT AND FUTURE

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Background: HCV infection prevalence was found to be unexpectedly high in transfusion dependent thalassemic children in 1998. Till then HCV screening could not be done in blood donors. By the year 2000, with JICA assistance HCV screening program initiated at National Blood Center.
Aims: To understand the effectiveness of HCV screening under National Blood Programme within 15 years of implementation.
Methods: A longitudinal study through hospital statistics and patients' registers anti-HCV positive rate was recorded.
Results: According to a Japan-Myanmar collaborative study conducted in 1998, the HCV infection among transfusion taking thalassemic patients at the Haematology Department of the Yangon General Hospital and at the Yangon Children's Hospital was found to be 55.5% and 46.7%, respectively. With the support of JICA, National Blood Center (Myanmar) could be able to introduce HCV screening of blood donors in year 2000. Since then many of Myanmar study groups continued monitoring the prevalence of HCV infection among multi-transfused patients, both children and adults. It was found to be declined gradually year by year as 12.6% and 4.8% in thalassemic adults and children respectively by 2010. According to the recent report in 2017, it declines further to 5.8% and 2.7% respectively.
Summary/Conclusions: This achievement of dramatic improvement of HCV positive rate among multi-transfused patients is one of the characteristic features of successful implementation of National Blood Programme in line with National Health Programme, National Blood Policy and Blood and Blood Product Law in Myanmar.

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HCV INFECTION IN PATIENTS WITH THALESSEMIA

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Background: In Iran, blood screening for Hepatitis C virus (HCV) has been implemented from 1996 and as a result, the risk of HCV infection has been decreasing in blood donors and blood recipients. Thalassemia patients need blood products for transfusion regularly.
Aims: This study was planned to investigate the prevalence of HCV infection among thalassemia patients.
Methods: In this cross sectional study 228 thalassemia patients referred to IBTO research center from Mar. 2013 to Sep.2013 were included. Plasma samples were tested for anti HCV by ELISA method. Reveres transcription polymerase chain reaction assay was done to confirm HCV infection in positive anti HCV test results. Data were analyzed by chi-square and *t*-test using SPSS version 16.
Results: Out of 228 thalassemia patients, 105(46.1%) were male. the mean age ± SD was 27.84 + /- 6.24 years. One hundred and eighty-one patients (79.4%, CI 74 to 84.8) were anti HCV positive. HCV RNA was detected in 40 patients (17.5%, CI 13.3 to 21.7) with or without antiviral therapy. No significant differences were found between HCV RNA positive patients and HCV RNA negative patients in sex and age.
Summary/Conclusions: To achieve a lower rate of HCV infection transmission, other risk factors for HCV infection should be considered.

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HIGH HCV INFECTION, HIGH SPONTANEOUS HCV CLEARANCE PREVALENCE AND BROAD GENOTYPE 6 DIVERSITY OF THE LI ELDER MINORITY (>60 YEARS) IN BAISHA COUNTY, CHINA

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Background: The epidemiology of hepatitis C virus (HCV) infection varies widely across geographic regions and ethnic groups. Previous study showed that the genotypes of HCV in Baisha county are genetically related but distinct from each other belonging to genotype 6.
Aims: The aim of this study was designed to understand the epidemiology of HCV in the Li minority in Baisha county, Hainan Province.
Methods: Anti-HCV and HCV RNA viral load were measured in all participants. Direct-sequencing of NS5B and E1 regions were used to determine the HCV genotype. Univariate chi-square test and multivariable logistic regression analysis were used to determine the risk factors for HCV infection and HCV spontaneous clearance.
Results: Among 1,682 participants, 117were anti-HCV positive (7.0%),while at least 42.7% of confirmed anti-HCV carriers had no detectable HCV RNA and were thus considered to have cleared HCV and recovered from the infection. Anti-HCV positive was associated with age (>60 years) (OR = 39.49, 95% CI 15.90–98.09, P < 0.01) and surgery (OR = 2.36, 95% CI 1.09–5.10, P = 0.03). The genotype distribution characteristic of Baisha county was unique, complex and diversity. They can't cluster with any assigned subtypes but form four Baisha community-specific groups (Figure 1).
Summary/Conclusions: HCV infection is characterized by high HCV infection, high spontaneous HCV clearance prevalence and broad genotype 6 diversity of the Li elder minority (>60 years) in this rural community.

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Abstract has been withdrawn.

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EVALUATION OF THE PERFORMANCE CHARACTERISTICS OF AN IN-HOUSE ONE STEP TAQMAN REAL TIME RT-PCR ASSAY FOR DETECTION AND QUANTIFICATION OF HEPATITIS C VIRUS

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Background: With the improvement of quantitative molecular HCV RNA assays the usefulness of these assays has been clarified for management of HCV infection. Recently, various real time assays with different methodology and performance characteristics have been introduced.
Aims: The aim of this study was to design, develop and evaluate an in-house one step TaqMan Real Time RT-PCR assay for detection and quantification of HCV-RNA.
Methods: The primers and probe were selected from highly conserved region of HCV genome, allowed detection of four common HCV genotypes in Iran. Using four quantification standards from 10^1 IU/µl to 10^4 IU/µl and clinical specimens, we determined analytical sensitivity, linear range, precision, analytical and clinical specificity and trueness of the assay. Data was analyzed by statistical software (SPSS, Version 16).
Results: The sensitivity of the assay with 95% probability determined by probit analysis was 15 IU/µl. The assay showed a linear range of 10^1 IU/µl to 10^4 IU/µl (R^2 =0.989). The coefficient of variation for intra and inter assay precision of the assay based on threshold cycle value ranged from 0.24 to 0.4 and from 1.94 to 3.19, respectively. The analytical and clinical specificity were 100%. No bias in relation to concentration between the results of 27 HCV RNA positive clinical specimens simultaneously tested by artus HCV LC RT-PCR reagents and in-house reagents was observed in method comparison.
Summary/Conclusions: The in-house one step TaqMan Real Time RT-PCR assay showed acceptable performance characteristics. Our study presents a robustness and cost- effective method for detection and quantification of HCV RNA.

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CONFIRMATORY HCV TESTING WITH TWO IMMUNOBLOT ASSAYS

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Background: According to the national algorithm for TTI testing, initially reactive samples are tested in duplicate by the initial screening test. Repeatedly reactive samples are subjected to further confirmatory testing in order to determine the future status of the blood donor.
Aims: The aim of the study was to assess two immunoblot assays on the repeatedly reactive blood donor samples by anti-HCV screening immunoassay.
Methods: The 52 repeatedly reactive blood donor samples with the Abbott anti-HCV immunoassay were subjected to confirmatory testing with two different immunoblot assays, the Deciscan HCV Plus and the RecomLine HCV in parallel. The obtained results were compared and analyzed. The result was considered as false positive by anti-HCV screening assay if the two immunoblots were negative. The result was positive if at least one of the immunoblots was positive and the result was considered indeterminate if at least one of the immunoblots was indeterminate.
Results: In 2016, 30,450 blood donor samples were screened for anti-HCV antibodies with Abbott Architect system out of which 64 (0.21%) were initially reactive and 52 (0.17%) of them were repeatedly reactive. Confirmatory testing with Deciscan HCV Plus revealed the following: 7 (13.5%) positive, 5 (9.6%) indeterminate (4 of them were HCV RNA positive by PCR) and 40 (76.9%) negative results. Confirmatory testing with RecomLine HCV revealed the following: 6 (11.5%) positive, 10 (19.2%) indeterminate (only 2 were HCV RNA positive by PCR) and 36 (69.2%) negative results. Concordant results were obtained with both tests in 41 (78.8%) of the samples: 6 (14.6%) positive, 2 (4.8%) indeterminate and 33 (80.5%) negative results. One sample was positive only by Deciscan HCV Plus, being indeterminate by RecomLine HCV. Based on results from both techniques, the total of 7 (13.5%) samples were considered positive, 12 (23.0%) were considered as indeterminate and 33 (63.5%) were considered as negative.
Summary/Conclusions: According to the frequency of indeterminate results and certain difficulties in interpretation of the results with RecomLine HCV assay, we found Deciscan HCV Plus immunoblot assay to be more suitable and reliable as HCV confirmatory test.

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GB VIRUS B CHIMERAS CARRYING WHOLE STRUCTURAL PROTEIN OR MAJOR NONSTRUCTURAL PROTEIN OF HEPATITIS C VIRUS ARE INFECTIOUS IN COMMON MARMOSETS

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Background: The development of vaccination and novel therapy for hepatitis C virus (HCV) has been hampered by the lack of suitable small primate animals. GB virus B (GBV-B) closely related to HCV causes viral hepatitis in common marmosets (*Callithrix jacchus*) might represent an attractive surrogate model for HCV infection. However, differences exist between GBV-B and HCV in spite of a short genetic distance between the two viruses.
Aims: To construct a suitable small primate animal model for studying HCV infection.
Methods: Three HCV/GBV-B chimeras containing HCV structural genes coding for either whole core and envelope proteins (CE1E2p7), or full envelope proteins (E1E2p7), or major nonstructural proteins (NS2NS3NS4A) substituted for the counterpart elements of GBV-B were constructed. Naïve animals and FK506-treated immunosuppressed marmosets were intra-hepatically injected with chimeric RNA transcripts or intravenously injected with sera from primary infected animals. Viral RNA in the serum and liver, adaptive mutations of chimeric virus, histopathological changes and viral protein expression in the liver, anti-HCV reactivity and virus-specific T cell immune response were measured.
Results: All three chimeras were infectious to the marmosets and caused chronic infection. The infected animals presented typical characteristics of viral hepatitis, including persistent circulating viremia, viral RNA and proteins in hepatocytes, histopathological changes in liver tissues. FK506-induced immunosuppression facilitated the establishment of chimera persistent infection. Chimera recovered from liver samples presented some adaptive viral mutations. Fluctuations of chimeric virus replication in marmosets with spontaneous and sporadic viral clearance might be related to specific antibody and T-cell response to HCV proteins.
Summary/Conclusions: The chimera infected marmosets described can be used as a suitable small primate animal model for evaluation of virus-cell interaction, vaccination and antiviral therapy against HCV infection.

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Abstract has been withdrawn.

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SEROLOGICAL REACTIVITY OF HCV INFECTED DONATIONS PRIMARILY NEGATIVE IN SEROLOGICAL SCREENING (NAT YIELDS)

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Background: The recent years have witnessed significant progress not only in HCV detection with NAT, but also with serological methods.
Aims: To assess the clinical sensitivity of different types of serological tests in donations primarily recognized as NAT yields in routine screening.
Methods: The study included 106 Polish donations from the early stage of HCV infection – negative in serological screening but positive in RNA HCV testing – identified in 2002–2016: 27 infected donations were identified with AmpliScreen HCV (Roche), 29 with Amplicor (Roche), 21 with Taqscreen MPX (Roche), 12 with Taqscreen MPX 2.0 (Roche), 3 with Procleix HCV/HIV-1, 9 with Procleix Ultrio (Novartis), 4 with Ultrio Plus (Novartis) and 1 with Utrio Ellite (Grifols). Out of 106 NAT yields 65 were originally negative in the HCV ELISA V3.0 (ORTHO), 25 in the Architect Anti-HCV (Abbott) and 16 in Vitros anti-HCV (Ortho). The average concentration of RNA HCV (VL) in the samples ranged from 6 to $4.78x10^7$ IU/ml ($2.3x10^6$ IU/ml on average, median $4.43x10^5$ IU/ml. Donations were infected with genotype 1a (4.7%), 1b (40.6%), 3a (45.3%) 4 (7.5%) and with mixed genotypes (1.8%). Sample reactivity was analyzed in 4 anti-HCV assays: in two chemiluminescence assays (test I – Vitros aHCV, test II – Abbott anti-HCV), in fourth-generation ELISA (test III – Bio-Rad Monolisa HCV Ag-Ab Ultra V2) and in electrochemiluminescence assay (test IV – Anti-HCV Roche Cobas II). In case of reactive result the test was repeated twice. Additionally, all samples were tested with HCV Ag test (test V – Abbott n = 70 or Ortho n = 36).
Results: 32.1% of all samples were found reactive in fourth-generation ELISA (all tested positive in assay V), and 11.34% were reactive in electrochemiluminescence assay (all tested positive in the assay V and eight donations in test III). One donation (0.94%) was repeatedly reactive in chemiluminescent assay (test I). VL was lowest in samples negative in all serological tests (assays I-V, n = 18, average VL = $2.2x10^5$ IU/ml) and anti-HCV positive in assay I and IV (n = 13, average VL-$9.3x10^5$ IU/ml), the highest VL was measured in samples tested positive in the fourth-generation assay (four tested positive, but negative in tests I, II and IV, n = 26, average VL = $3x10^6$ IU/ml). The differences were statistically significant.
Summary/Conclusions: In the comparative evaluation study of donations primarily identified as RNA HCV yields the highest clinical sensitivity is presented by fourth-generation ELISA, and then electrochemiluminescence anti-HCV assay.
Authors thank Roche Diagnostics Poland and Bio-Rad Poland for support in realization of the study.

HIV

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MOLECULAR EPIDEMIOLOGICAL OF HIV-1 VIRUS AMONG BLOOD DONORS IN MALAYSIA

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Background: The prevalence rate of HIV-1 infection among blood donors in Malaysia is 0.04% over 8-year period (2008–2014) at 1 HIV-1 infected donors per 2,600 donation. This may poses a major risk for unsafe blood transfusion due to window period phase or diversity of HIV-1 virus that may escape current screening assay. Implementation of nucleic acid testing (NAT) at National Blood Centre Kuala Lumpur in 2008 has greatly reduce the residual risk transmission from 8.99 to 2.04 per million donations. Few recent studies had observed newly emergence HIV-1 circulating recombinant forms (CRFs) in high-risk populations in Malaysia including people who inject drugs (PWID).

Aims: Given the recent emergence of several HIV-1 circulating recombinant forms (CRFs) in high-risk populations in Malaysia, we investigated the genotypic distribution of HIV-1 among the blood donor population in National Blood Centre Kuala Lumpur. This survey is the first to document and characterize HIV-1 genetic variation and epidemiological profiles in Malaysian blood donor.

Methods: A total of 460,060 donations were screened at the National Blood Centre of Kuala Lumpur between 2013 and 2014 using HIV O Plus assay (Abbott Laboratories) and Procleix Ultrio Plus assay (Grifols Diagnostic Solution). During this period, 211 plasma samples were confirmed to be HIV-1 positive by serology or NAT or both. HIV-1 RNA was extracted from plasma before HIV-1 *gag-pol* genes were amplified and sequenced followed by genotype determination using phylogenetic and recombination analyses.

Results: Out of 211 samples processed, only 149 partial *gag-pol* gene sequences were successfully amplified. Major circulating HIV-1 genotypes determined by neighbor-joining phylogenetic inference included CRF01_AE at 40.9% (61/149), CRF33_01B at 21.5% (32/149), and subtype B at 10.1% (15/149). Newly-described CRFs including CRF54_01B circulated at 4.0%, CRF74_01B at 2.0%, and CRF53_01B and CRF48_01B at 0.7% each. Interestingly, unique HIV-1 genotypes including African subtype G (8.7%), CRF45_cpx (1.3%), CRF02_AG (0.7%) and CRF07_BC (0.7%) from China were detected for the first time in the country. A cluster of subtype G sequences formed a distinct founder sub-lineage within the African strains. In addition, 8.7% (13/149) of HIV-infected donors had unique recombinant forms (URFs) including CRF01_AE/B' (4.7%), B'/C (2.7%) and B'/G (1.3%) recombinants.

Summary/Conclusions: Molecular epidemiological studies provide an effective strategy to detect the major circulating HIV-1 strains in infected blood donors, as well as to detect newly-emerging or unique viral Strains. Multiple introductions of HIV-1 genotypes observed in this study from high prevalent country like Africa and China alarm us about the molecular complexity of HIV-1 virus among Malaysian blood donor. This molecular finding is important to ensure no virus can escape blood screening strategy thus provide safe blood to the patient. Continuous molecular surveillance of HIV-1 among blood donors is crucial and more samples should be use from other blood center to get a complete epidemiological data for HIV-1 virus nationwide.

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HIV POSITIVITY RATIO AMONG BLOOD DONORS IN BLOOD BANK OF ULUDAG UNIVERSITY FACULTY OF MEDICINE, BURSA, TÜRKIYE

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Background: It has been shown that HIV incidence has increased in our country in the course of time. We thought that this increase could be parallel with HIV positivity among blood donors. For this reason we planned to investigate and compare confirmed HIV positive results of our center with national results.

Aims: We aimed to detect the impact of HIV incidence increase seen in society on blood donor population.

Methods: We searched confirmed HIV positive test results among blood donors from blood bank records, retrospectively. Date interval of this study was between 01.01.2005–31.12.2016. We chose this 12 years interval because of our first confirmed case found in 2005. All HIV tests were studied with ELISA, specific for anti-HIV I/II antibody and p24 antigen. Tests were run with Microparticle Enzyme *Immunoassay* (Abbott-Axsym System) until February 2013 and run with chemiluminescent microparticle *immunoassay* (Abbott-Architect Plus i1000) from these date to end of the study period. All samples that were repeatedly reactive confirmed with western blot method in national reference laboratory.

Results: We detected 13 confirmed HIV positivity between 2005–2017 in 245.349 blood donation. HIV positivity ratio has been calculated as 53 in one million donation. Our results have been shown in Figure-1. Confirmed new HIV positivity numbers in our country with regard to years from 2005 to 2016 (first six month) has been shown in Figure-2. It has been analyzed that confirmed HIV positivity trends between blood donors and national results have had statistically significant relationship ($P < 0.05$).

Summary/Conclusions: Confirmed HIV positivity in our blood donors has been increased especially in last two years similar with national data. Despite our overall positivity ratio was calculated as 0.0053% for years between 2005–2016, this ratios were found as 0.0133%, 0.0083% and 0.0168% in years 2011, 2015 and 2016, respectively (Figure-1). According to these results, it is possible to say that increased HIV positivity in society might have affected the blood donor population. But confirmed HIV positivity rate in blood donors is still lower than general population. This difference may be a result of blood donor population awareness about risks and success of donor selection process.

As a result, although we don't have a great number of confirmed HIV positivity, our positivity number gradually increase. For more acceptable results, we need a greater number of multicentral studies and data.

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HIV POSITIVE DONATIONS AT NATIONAL BLOOD CENTRE, KUALA LUMPUR, MALAYSIA

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Background: The National Blood Centre, Kuala Lumpur (NBCKL) has been routinely screen all their collected blood donations using both serology and individual-donation nucleic acid testing (ID-NAT) method since November 2007. HIV-1 infected donations have been detected thru both screening method.

Aims: To determine the prevalence and residual transmission risk of HIV-1. To study HIV positive donations detected from both serology and ID-NAT routine screening.

Methods: For HIV-1 serology screening, samples were screened with Abbott Prism Anti-HIV. Serology repeatedly reactive samples will then be subjected to EIA 2 test (Roche Cobas 6000, HIV Ag-Ab combo), supplementary test (PA-HIV 1/2, Fujirebio) and confirmatory test by LIA HIV (Fujirebio). ID-NAT screening was performed on Tigris platform (Grifols, Ultrio Plus) and if found to be reactive, will proceed to discriminatory test (dHIV). Potential HIV-1 NAT yield (dHIV detected, seronegative) were sent for quantitative PCR test (Roche Taqman) if budget permitted. The prevalence rate of HIV-1 was calculated. The residual transmission risk for HIV-1 among repeat and lapsed donations was estimated using the refined mathematical model of Weusten *et al.*2011.

Results: Between years 2008 – 2016, a total of 1,439,985 donations were screened (596,335 for first time donations and 843,650 from lapsed+repeat donations). The HIV-1 prevalence rate among NBCKL blood donors was 0.04%. The residual transmission risk for HIV-1 was estimated at 1.97 per million donations (1 in 507,042 donations). 626 (95.69%) HIV-1 infections were interdicted, where 27 (4.31%) were HIV-1 NAT yields (1: 53,333). Quantitative PCR were performed on 25 HIV-1 NAT yield samples and seven were detected with viral load >100,000cps/ml. Sixteen HIV-1 NAT yield donors who came back for counselling as early as two weeks from index donation all developed antibody to HIV-1. Confirmatory LIA for the fifteen out of sixteen counselling samples found that fourteen were positive and one negative but NAT remained detected. The earliest period of HIV serology confirmed positive is 26 days from index donation.

For HIV-1 serology and ID-NAT concordant donations, 387 (64.72%) were new donations and 211 (35.28%) were from lapsed and repeat donations. However for HIV-1 NAT yields, the majority were from lapsed and repeat donations (twenty lapsed+repeat donors (74.07%), while seven (25.93%) from new donors). The male gender dominated the HIV-1 positive donors group (95.95%).

Summary/Conclusions: Implementation of both serology and ID-NAT as routine screening for donation samples greatly enhanced blood safety. The percentage of HIV-1 NAT yields among all HIV-1 infection might be small (4.31%), but is enough to cause serious damage in blood transfusion service by infecting all recipients who received the tainted blood products with HIV-1 infection. No elite controllers were detected. Regular donors were generally viewed as safer than new donors. However, in NBCKL's scenario, majority of HIV-1 NAT yields were from regular donors, and majority of all HIV-1 infected donors were male. Further study need to be conducted on them, seeing as they are usually perceived as familiar and aware of blood donation criteria. Pre-donation screening should be strengthened to prevent those with high risk from donating in future.

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Abstract has been withdrawn.

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DEMOGRAPHIC CHARACTERISTICS OF HIV SEROPOSITIVE BLOOD DONORS – 10 YEARS STUDY

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Background: The availability of sufficient quantities of safe blood is the main goal of transfusion services as well as medical institutions that use blood and blood components in the treatment of patients. The risk of acquiring a transfusion-transmissible infection (TTI) from a blood component today is lower than ever. Continuous improvement in donor selection, screening tests and inactivation procedures can reduce the risk of acquiring TTI.
Aims: To present demographic characteristic of HIV seropositive blood donors in the South Backa District of Vojvodina.
Methods: A retrospective descriptive study was carried out in the Blood Transfusion Institute of Vojvodina from January 2007 to December 2016. The study used data of screening and confirmation tests for HIV infection from the records of the TTI laboratory. Blood donors who were confirmed positive were analyzed regarding to sex, age and the number of blood donations.
Results: A total of 300,484 blood donations were collected during ten years. The number of repeat blood donors has shown decreasing trend, while the number of first blood donors was stable. The overall seroprevalence of HIV infection was 0.0023% (ranged from 0 to 0.0108%). Five years have passed without HIV seropositive blood donors, in four years we had one seropositive blood donor per year, so the last year three blood donors were confirmed as HIV seropositive. The HIV infection was more prevalent in male repeat blood donors, aged 18 to 39 years.
Summary/Conclusions: This study reflects that the overall seroprevalence of HIV infection has been low. The risk of HIV infection was increased over time, so blood transfusion remains a risk factor for the spread of blood-borne infections. Therefore, it is necessary to increase the blood safety in our District. Thus, the collaboration of all parties involved in transfusion chain, including national hemovigilance system, is crucial.

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PERFORMANCE OF NEW AUTOMATED IMMUNOASSAY ASSAY FOR HIV ON THE ALINITY S SYSTEM

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Background: Every blood donation is screened to detect the presence of antibodies, or the presence of antibody and antigen, to human immunodeficiency virus type 1 and 2 (anti-HIV-1/2). Blood centers require very high throughput anti-HIV-1/2 assays with high specificity and sensitivity to prevent unnecessary donor deferrals while maintaining a safe blood supply. In addition, continued pressures on laboratory operations demand that assays perform on platforms capable of increased walk away time and enhanced automation in areas of reagent management, retest options, and commodity/waste management. In the response for the need for such screening assays, we have evaluated an improved automated assay for the detection of anti-HIV-1/2 antibodies and HIV-1 p24 antigen.
Aims: To evaluate the overall performance of a new chemiluminescence combo immunoassay for the detection of anti-HIV-1/2 antibodies and HIV-1 p24 antigen on a next generation automated platform, Alinity s.
Methods: The performance of the new chemiluminescence immunoassay for the detection of anti-HIV-1/2 antibodies and HIV-1 p24 antigen was evaluated on the Abbott Alinity s platform. Precision was assessed over 20 days evaluating positive samples. Specificity was evaluated on samples obtained from random blood donors and plasmapheresis donors. Sensitivity was evaluated using presumed positive samples for HIV-1, HIV-2 and HIV Group O antibodies and HIV-1 p24 antigen. Seroconversion sensitivity was evaluated with 41 commercial seroconversion panels.
Results: Precision was less than 8% CV for positive samples over 20 days. The blood donor specificity was 99.95% (6,226/6,229). Sensitivity was 100% for 427 presumed antibody positive samples comprised of HIV-1, HIV-2 and HIV-1 groups O, N, P, CRF and URF samples. Also, sensitivity was 100% for 102 antigen positive viral isolate samples comprised of HIV-1, HIV-2 and HIV-1 groups O, N, P, CRF and URF samples. Seroconversion detection was as good as the comparator assay with 136 reactive samples detected with the Alinity s assay and 135 reactive samples detected by the comparator assay.
Summary/Conclusions: These results indicate that the new automated Alinity s HIV Ag/Ab Combo assay provided very good performance in specificity, sensitivity and precision.

Bacteria

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SUSPENDING POOLED PLATELETS IN PLATELET ADDITIVE SOLUTION (PAS)- WHAT IS THE IMPACT ON NUMBERS AND SPECIES DETECTED BY BACTERIAL SCREENING?

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Background: Since mid-2015, pooled platelets manufactured by NHS Blood and Transplant (NHSBT) have been suspended in Platelet Additive Solution (Macopharma SSP+). This was introduced as a vCJD safety measure in parallel with the removal of the recommendation to collect >80% of platelets by apheresis. The available literature suggested that suspension of platelets in PAS would have no detrimental impact on bacterial screening results and may lead to an increase in detection sensitivity. Following implementation, the clinical team reported a change in frequency and type of species detected which required further investigation.
Aims: To review the impact of manufacturing pooled platelets in PAS on bacterial screening detection rates and type and frequency of microorganisms detected.
Methods: NHSBT has routinely screened all apheresis and pooled platelets for the presence of bacteria since 2011 using the BacT/ALERT system. The introduction of pooled platelets (from 4 donations) in PAS was rolled out between February and June 2015. All positive screening results are reviewed by the clinical team with donor follow-up as appropriate. A surveillance system was put in place when screening commenced, Initial reactives and results of confirmatory tests are routinely collected Including numbers and species of bacteria confirmed positive. Bacterial screening results from July 2013 to December 2014 pre-PAS (period A) and post-PAS introduction (period B, July 2015 to December 2016) were reviewed for the percentage of packs confirmed positive and the frequency of species detected.
Results: Pooled platelets comprised 25% of all platelet packs screened in period A and 41% in period B, only data on pooled platelets are reported here. During period A 105,034 packs were screened and 292 (0.28%) were initially reactive, of the total screened, 0.06% (95% CI 0.05%>0.08%) were confirmed positive. During period B 178,350 packs were screened, 331 (0.19%) were initially reactive and 0.08% confirmed positive (95% CI 0.06%>0.09%). There was no significant difference ($P > 0.05$) in the numbers confirmed positive in the two periods. Both pre- and post-introduction of PAS, the majority of isolates were identified as *Propionibacterium* spp. or *Staphylococcus saccharolyticus* (56/68 v 34/100 respectively). The percentage of 'other' bacteria increased from 17.6% (95% CI 9.5–28.8%) to 25.4% (95% CI 18.3–33.6%) although this was not significantly different. Potentially pathogenic organisms isolated pre-PAS included one each of *S. aureus*, *Pseudomonas aeruginosa*, *Streptococcus agalactiae*, and *S. pneumoniae*. Post PAS, additional species were observed, including potentially pathogenic organisms such as *Bacillus cereus*, although this change may just reflect infrequent events. Post PAS, the proportions of both *S. epidermidis* and Group C/G streptococci increased from 1.4 to 6.0%.

Summary/Conclusions: Following the introduction of pooled platelets suspended in PAS, the clinical team noted an increase in the number of platelet packs positive for Group C/G Streptococci and other, more unusual organisms. The overall rate of confirmed positive pooled platelets has remained stable since screening was introduced and although the frequency of potentially harmful species of bacteria has increased post-PAS, this is not statistically significant. Further work is required to understand the significance of this change.

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BACTERIAL SCREENING OF PLATELETS BY 16SR PCR

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Background: Bacterial contamination of platelet concentrates remained as an important threats for blood safety. Especially it is more important for transfusion to the immunocompromised recipients. No bacterial screening methods has been established in Iranian Blood Transfusion Organization yet.
Aims: present study investigate the bacterial contamination rate among Platelet concentrates in Tehran Blood center by implementing 16s rDNA PCR method.
Methods: Totally 1,500 platelets concentrate were randomly selected and studied by 16s rDNA PCR. The samples were taken from PCs at different time of storage. DNA extraction was done by QIA gene DNA extraction kit. The positive control of PCR was run by using Staphylococcus Aureus as control for gram positive bacteria and Escherichia coli as control for gram negative bacteria, negative control was also run concomitantly. Standard homemade (Takapoo zist) primers was used as PCR template and was added to the PCR master mix (Amplicon). The presence of bacteria was subsequently investigated by gel electrophoresis on the PCR products. The positive products further studied by DNA sequencing.
Results: Of the 1,500 PCs, one was found contaminated by a gram negative bacteria. By further investigation it was confirmed that the responsible organism was Pseudomona Aeruginosa. It was isolated on day 4th of incubation. No evidence of infection in donor was found by reviewing the donor questionnaire.
Summary/Conclusions: The prevalence of bacterial contamination is decreased apparently in TBTC.
16rsDNA PCR could be used as a useful rapid method for bacterial screening.

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SENSITIVE IMMUNOASSAY FOR THE EARLY AND GENERIC DETECTION OF BACTERIA IN PLATELET CONCENTRATES

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Background: Bacterial contamination of platelets is the highest residual infectious risk in transfusion despite the current preventive strategies. While bacterial contamination may affect any blood component, the ambient storage temperature conditions for platelets make them most likely to facilitate bacterial growth. Based on all the precautionary measures, the final platelet concentrates include in the worst cases a very limited viable bacteria number estimated from 10 to 100 colony forming units (CFUs)/bag (i.e. 0.03 to 0.3 CFU/ml). One major difference between viruses and bacteria is that bacteria have the ability to grow up to a concentration of 10^8–10^9 CFU/ml over the 5 days product shelf-life. Moreover, a large diversity of strains is found in contaminated platelets representing a key challenge for the development of a generic bacterial test. Many countries have implemented culture methods for bacterial screening with a negative-to-date concept. Although this system has a high sensitivity, most platelet concentrates are already transfused before a reactive signal occurs. To overcome this issue, rapid tests are recommended on Day 4 and Day 5 after collection as a safety measure. Furthermore, different pathogen reduction methods were developed but disadvantages might be their high costs and limited efficiency on spores and rapid growing bacteria.
Aims: In this study, we aimed to develop an economic and easy diagnostic approach for the early, rapid, sensitive and generic detection of bacteria in platelet concentrates. The adaptability of the process developed here with the blood transfusion services requirements was of major concern. Hence, our attention was focused on an easy to automate technique able to deliver definitive results on Day 2 after collection.
Methods: A large panel of bacteria involved in transfusion reactions including clinical isolates and reference strains (including the ISBT reference panel) was established and used for mouse immunizations, antibody screening and platelet spiking steps. An original approach was used to produce and select monoclonal antibodies directed either against Gram negative or against Gram positive bacteria to develop our generic immunoassay. As recommended, 24 h (Day 1) after collection a sampling volume of spiked platelets (0.1–1 CFU/ml) was tested after a short generic culture, lysis and capture of bacteria on magnetic microparticles in a microplate format. An immunoassay was performed for the detection of the captured bacteria.
Results: This approach was tested on a large panel of bacteria involved in transfusion reactions. The pre-analytical steps and the capture of bacteria on microparticles were improved to avoid false negative results and to enhance the sensitivity of detection. The full test developed in this study combining a pre-analytical culture step followed by an immunoassay easy to automate allows a sensitive detection of 10 CFU/ml for all Gram negative bacteria tested and 10^2 CFU/ml for Gram positive ones.
Summary/Conclusions: In this study, a new approach of rapid bacterial culture followed by microplate immunoassay was developed for the generic and sensitive detection of bacteria in platelet concentrates, able to be easily implemented in transfusion services to deliver tested platelets as soon as on Day 2 after collection. This original approach could be adapted for the bacterial detection of other blood products.

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THE MOST COMMON ISOLATES OF MICROBIOLOGICAL ENVIRONMENTAL MONITORING IN THE BLOOD PRODUCTION DEPARTMENT IN THE CROATIAN INSTITUTE OF TRANSFUSION MEDICINE

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Background: Microbiological environmental monitoring (EM) is carried out in the Blood Production Department regularly, according to defined plans and methods, including all the equipment and hands of the employees who work in these areas. EM provides system controls, assesses the effectiveness of cleaning/disinfection and increases the safety of blood components. Critical samples are considered: head of tube sealer, clamps of sterile connection device and plasmatherm interior surface.
Aims: The aim is to show our five-year EM results, both number and microbial isolates from January 2012 to December 2016.
Methods: All flat surfaces were sampled using contact plates. Cotton swabs were used in places where contact plates were not applicable. Hygienic behavior control of employees was performed by a fingertip impression technique using Tryptic soy agar. The Swabs were inoculated on Columbia blood agars. Bacteria colonies per plate were counted and identified using phenotypic methods.
Results: During the five-year environmental monitoring period in the Blood Production Department 5,724 samples were collected and processed. Coagulase-negative staphylococci were the most common isolates, found in 61.42% of samples (3,516/5,724). *Staphylococcus aureus* was isolated in 0.8% of samples (47/5,724), *Micrococcus spp* in 49.37% (2,826/5,724) and *Streptococcus spp* in 0.04% (2/5,724). *Bacillus spp* was isolated in 25.45% of samples (1,457/5,724), diphtheroids in 10, 60% (607/5,724), different molds grew in 2.9% (166/5,724). Nonfermenting gram negative rods were isolated in 2.62% of samples (150/5,724), of which *Acinetobacter spp* in 104 samples (Acinetobacter *spp*. 81/104, Ac. lwoffi 15/104, *Ac. radioresistens* 5/104, Ac. baumannii 2/104, *Ac. jejunnii* 1/104), *Pseudomonas spp*. 24/150 (not aeruginosa), *Agrobacterium radiobacter* 5/150, *Brevundimonas vesicularis 1/150, Chryseobacterium indologenes 1/150, Stenotrophomonas maltophilia 3/150, Ralstonia pickettii 4/150, Sphingomonas paucimobilis 3/150, and other Gram negative nonfermentors 5/150. Members of enterobacteria were isolated only in 0.14% of samples (8/5,724) including Pantoea spp in 7, Klebsilla spp in 1, and Enterobacter cloacae in two samples. Nocardia spp was isolated in six samples, and Ochrobactrum anthropi* in six samples. Growth above the action level was found in 4.68% of critical samples altogether: tube sealer combined with sterile connection device samples (30/520) and plasmatherm samples (31/779).

Summary/Conclusions: Generally, the most common EM finding was a mixed microbial population and the most common isolates were coagulase-negative staphylococci, *Micrococcus* spp, *Bacillus* spp, diphtheroids and molds. Gram negative rods (nonfermentors and enterobacteria) were isolated less frequently. Tube sealer and the sterile connection device samples mostly had no microbial growth. The samples with low bacterial growth could be explained with long inactivity periods. Plasmatherm sampling mostly presented satisfactory results, with *Pseudomonas* spp as a more common isolate, the latter not being surprising since its inclination to moist areas.
Monitoring and analysis of trends and knowledge of "in-house" strains allows continuous system control and cleaning/disinfection procedures, consequently performing preventive and corrective measures in case of adverse events in terms of large numbers of new microbes or new species.

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PERFORMANCE OF A NEW AUTOMATED IMMUNOASSAY FOR THE DETECTION OF SYPHILIS ON THE ALINITY S SYSTEM

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Background: Blood donations are commonly screened for Syphilis in order to detect the presence of antibodies to the bacterium *Treponema pallidum*. In addition, continued pressures on laboratory operations demand that assays perform on platforms capable of increased walk away time and enhanced automation in areas of reagent management, retest options, and commodity/waste management. In response to the need for increased specificity for Syphilis screening assays, we have evaluated an improved automated assay for the detection of antibodies to *T. pallidum*.
Aims: Performance of the new automated chemiluminescence immunoassay for the detection of antibodies to *Treponema pallidum* was evaluated on the Alinity s system.
Methods: Precision was assessed over 20 days using positive samples. Specificity was evaluated on samples obtained from 6,393 blood donors from the US and Europe and 200 diagnostic samples obtained from the US. Sensitivity was evaluated on 400 preselected positive samples. Sensitivity and specificity samples were split across three reagent lots during testing. Confirmation of repeatedly reactive samples was performed using an algorithm with three recombinant immunoblot assays, INNO-LIA™ Syphilis Score, Mikrogen Diagnostik's *recom*Line Treponema IgG, and *recom*Line Treponema IgM blots.
Results: Precision was less than 6.0% CV for positive samples over 20 days. Clinical sensitivity was 100.00% (400/400) on preselected Syphilis positive samples. The specificity was 99.95% (6,354/6,357) on a blood donor population and 100.00% (200/200) on diagnostic samples.
Summary/Conclusions: These results indicate that the new automated Alinity s Syphilis assay provided acceptable performance in precision, specificity and sensitivity. Sensitivity and specificity were comparable to the comparator assay.

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Abstract has been withdrawn.

P-370

EVALUATION OF RAPID SCREENING TESTS FOR SYPHILIS IN ISLAMABAD, PAKISTAN

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Background: Diagnosis of syphilis through rapid screening assays provides the opportunity to prevent the disease to transmit through blood transfusion. Reliability and cost effectiveness should be key to use them for blood screening purpose. In resource limited countries where laboratory facilities are not routinely available, primary syphilis screening depends on the use of rapid point of care devices. These rapid point-of-care devices must be evaluated for diagnostic effectiveness before their use.
Aims: To evaluate the performance and diagnostic effectiveness of four assays for the diagnosis of syphilis in comparison with ARCHITECT i1000SR syphilis TP assay, a chemiluminescent microparticle immunoassay, and to propose a reliable and cost effective testing strategy for the diagnosis of syphilis in Pakistan.
Methods: This single centre prospective study was conducted at the Department of Blood Transfusion Services, Shaheed Zulfiqar Ali Bhutto Medical University, Islamabad from January – June 2016. A total of 240 specimens (serum) from Pakistani population were selected for evaluation. Out of 240, 100 blood serum were collected from suspected individuals in internally displaced persons (IDP) and Afghan refugees' camps in KPK. Four commercially available rapid point of care devices (SD Bioline syphilis 3.0, Rapidan Tester, Accurate, Acu-Check) were used to detect syphilis and result was compared with Architect syphilis TP assay (CLIA). Six parameters (sensitivity, specificity, positive predictive value, negative predictive value, positive diagnostic likelihood ratio, negative diagnostic likelihood ratio) were assessed to check the diagnostic effectiveness.
Results: 90/240 (37.5%) of the samples were positive for antibodies against treponemal antigen. The sensitivities of Accurate, SD Bioline syphilis 3.0, Rapidan Tester and Acu-Check remained 80% (95% CI; 70.25–87.69%), 70% (95% CI; 59.43–79.21%), 83.33% (95% CI; 74–90.36%) and 96.67% (95% CI; 90.57 to 99.31%) respectively. Accurate and SD Bioline syphilis 3.0 exhibited specificities of 94% (95% CI; 88.92 to 97.22%) and 92% (95% CI; 86.44–95.80%) respectively. Both Rapidan Tester and Acu-Check exhibited the specificity of 98% (95% CI; 94.27–99.59%). PPV values for Accurate, SD Bioline syphilis 3.0, Rapidan Tester and Acu-Check remained 88.89% (95% CI; 79.95–94.79%), 84% (95% CI; 73.72–91.45%), 96.15% (95% CI; 89.17–99.20%) and 96.67% (95% CI; 90.57–99.31%) respectively. NPV values for the same tests remained 88.68% (95% CI; 82.70–93.15%), 83.64% (95% CI; 77.09–88.93%), 90.74% (95% CI; 85.19–94.72%) and 98% (95% CI; 94.27–99.59%) respectively. Positive diagnostic likelihood ratios for Accurate and SD Bioline syphilis 3.0 were 13.33 (95% CI; 7.02–25.33) and 8.75 (95% CI; 5–15.31) respectively. Rapidan Tester and Acu-Check exhibited PDLR values of 41.67 (13.54–148.22) and 48.33 (95% CI; 15.76–148.27) respectively. Negative diagnostic likelihood ratios for Accurate, SD Bioline syphilis 3.0, Rapidan Tester and Acu-Check remained 0.21 (95% CI; 0.14–0.32), 0.33 (95% CI; 0.24–0.45), 0.17 (95% CI; 0.11–0.27) and 0.03 (95% CI; 0.01–0.10) respectively.
Summary/Conclusions: Acu-Check with high sensitivity and specificity compared to other three rapid assays could be used for diagnosis of syphilis in Pakistani population and it is also cost effective.

Parasites

P-371

IN VITRO CONFIRMATION OF THE PRESENCE OF ANTI-TRYPANOSOMA CRUZI ANTIBODIES IN HUMAN SERUM AND PLASMA SAMPLES

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Background: The Elecsys® **cobas**® **e** Chagas assay (detection of antibodies against Trypanosoma cruzi) is intended for use as a screening assay in blood banks as well as for diagnostic use. The assay uses three soluble recombinant *T. cruzi* antigens to detect specific antibodies in human serum and plasma. Due to the high dilutional sensitivity of the double-antigen sandwich format of the assay, discrepant reactive results might appear if compared to less sensitive serological methods. Antigen detection methods of *T. cruzi* do not always help to further assess the status of a patient of question. Different algorithms exist to verify or falsify the presence of anti-*T. cruzi* antibodies using different immunological methods and their respective sensitivities. Since serology is most important in the diagnosis of Chagas disease, a simple confirmatory method using native antigen-extract of *T. cruzi* to neutralize specific antibodies might add value in resolving discrepant findings.
Aims: Neutralization with native T. cruzi antigen is used as an in house confirmatory method to assess the presence of specific anti-T. cruzi antibodies in the sample of question. The proof of concept of this method will be demonstrated.
Methods: Confirmed serologically reactive Chagas samples (WHO-Antibody Standards, Ring Trial samples and patient samples) were derived from commercial vendors or clinical institutions. Native T. cruzi antigen extract was prepared from

lyophilized T. cruzi cells or was provided by reference institutions. Discrepant reactive samples of interest were split into two aliquots. One aliquot was pre-incubated in the presence of native antigen extract of T. cruzi and was subsequently run together with the untreated sample (reference) in the Elecsys Chagas assay. Recovery of concentration was calculated and a recovery of ≤ 25% of the initial (untreated) concentration was rated as successful neutralization.
Results: Neutralization was shown to be specific and applicable for high-antibody levels as well as for samples with low-levels of anti-T. cruzi antibodies. Neutralization could be achieved using antigens of different T. cruzi strains and origin.
Summary/Conclusions: The Neutralization by addition of native antigen extract of T. cruzi to the sample in question was shown to be a reliable and specific method for confirmation of the presence of anti-T. cruzi antibodies. The advantage of this simple method is the use of a heterologous antigen (if compared to the antigen used for detection in the respective assay) to generate a competitive situation for the specific antibodies. The proof of concept could be demonstrated and neutralization based on the use of T. cruzi antigen extract derived from different sources was shown to be specific.

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PERFORMANCE OF AN AUTOMATED IMMUNOASSAY FOR THE DETECTION OF TRYPANOSOMA CRUZI-SPECIFIC ANTIBODIES IN A NON-ENDEMIC COUNTRY

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Background: Chagas disease (CD) is caused by Trypanosoma cruzi (T. cruzi), a protozoan parasite usually transmitted by infected triatomine bugs. Transmission also occurs through transfusion or organ transplantation, congenital transmission, laboratory accidents, and rarely by ingestion of contaminated food or drink. CD is one of the most important endemic diseases in Latin America (LA), where approximately 6 million people are estimated to be infected, with about 12,000 deaths per year typically due to chronic heart disease or, less frequently, megadisease or meningoencephalitis. Because of population migration from endemic areas, clinicians and blood banks in the non-endemic countries are likely to see an increasing number of patients and donors with suspected or confirmed CD. CDC estimates that more than 300,000 persons with T. cruzi infection live in the United States, while a 2015 study indicates that 4.2% of the overall LA population living in Europe is affected by CD. Europe is currently hosting around 4 million of migrants from LA, especially in southern European countries such as Spain and Italy, followed by France and United Kingdom. Nevertheless, this initial distribution is changing due to the economic crisis and currently there is a redistribution or dispersion of LA migration, especially from South Europe to other European countries. The diagnosis of CD relies on different approaches, depending on the phase of the infection. During the chronic phase, parasitaemia is usually undetectable and inconstant. Direct parasitological methods or PCR are not, therefore, helpful in routine diagnosis, while serology is considered the best option. Today new generation tests, employing a large mixture of recombinant antigens, are showing improved accuracy compared to conventional assays using a whole parasite antigen, for which a well-known problem is cross reaction with antibodies produced by other pathogens, especially Leishmania spp.
Aims: To evaluate the performance of a fully automated assay made with recombinant antigens, on different populations of a country non-endemic for CD, and endemic for Leishmaniosis.
Methods: More than 5,200 blood donors, 239 pregnant women and 500 hospitalized patients samples, taken from the laboratory routine, have been tested with the LIAISON® XL Murex Chagas (DiaSorin S.p.A., Italy), an indirect chemiluminescence immunoassay (CLIA) made with a multi-epitope recombinant multi-antigen specific for T. cruzi on the solid phase and a mouse monoclonal antibody to human IgG, linked to an isoluminol derivative, as conjugate. Reactive results have been further investigate using additional methods, to establish a confirmed result.
Results: The observed diagnostic specificity of the assay on the blood donors was 99.96% (5,242/5,244), with 95% confidence limits of 99.86% to 100%, while the assay showed diagnostic specificity of 100% on hospitalized and pregnant women populations.
Summary/Conclusions: Chagas disease is emerging in European countries due to the migration flows from LA endemic countries and there is an urgent need to standardise, expand and reinforce the control measures against CD transmission. An accurate serological test, with high level of specificity, even on a population potentially cross reacting, may represents a useful tool for screening in blood banks and for routine diagnosis in clinical laboratories.

P-373

MOLECULAR SCREENING FOR MALARIA AMONG BLOOD DONORS IN A WHO CLAIMED REGION OF EGYPT, FAYOUM GOVERNORATE

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Background: Transfusion transmitted malaria is undoubtedly a potential hazard for blood recipients. Egypt is still in the *prevention of reintroduction* phase of malaria control program. Fayoum Governorate for its geology is considered one of the high risk foci in Egypt, however no studies have been reported to evaluate the current status of subclinical *Plasmodium* infection based on sensitive molecular techniques. Moreover, screening of malaria is not listed within screening protocols of blood-borne pathogens in Fayoum blood transfusion centers.
Aims: To assess the current prevalence of subclinical *Plasmodium* infection in Fayoum inhabitants in general, and among blood donors in particular for transfusion biosafety.
Methods: A cross sectional survey was conducted on 400 apparently healthy blood-donors in blood transfusion center of Fayoum University hospital. PCR was used to detect the 18 S ssrRNA *Plasmodium* gene.
Results: All Fayoum inhabitants' blood donors' samples were negative for *Plasmodium* infection.
Summary/Conclusions: Current applied control and preventive measures are effective in the context of blood transfusion biosafety in Fayoum blood banks and, therefore, the implementation of a routine malaria screening test in Fayoum blood banks is not merited at this time. In light of our study, we can assume that malaria has been successfully eliminated, at present, from the high risk foci in Egypt, Fayoum Governorate. However further comprehensive study is recommended to screen for anti-plasmodium and stratify the results according to age, to substantiate the assume eradication of the infection. Regular monitoring is still needed.

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Abstract has been withdrawn.

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PERFORMANCE OF A NEW AUTOMATED ASSAY FOR ANTIBODIES TO *T. CRUZI* ON THE ALINITY S SYSTEM

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Background: The parasite, *Trypanosoma cruzi* (*T. cruzi*), is the cause of Chagas disease which is endemic to the Americas and infects 6–8 million people. In order to prevent transfusion mediated transmission of this parasite in endemic countries, blood collection centers require high throughput anti-*T. cruzi* assays with good specificity and sensitivity. In non-endemic countries, selective testing of at risk donors is a strategy to avoid temporary donor deferrals. In addition, continued pressures on laboratory operations demand that assays perform on platforms capable of increased walk away time and enhanced automation in areas of reagent management, retest options, and commodity/waste management.

Aims: To evaluate the overall performance of a new chemiluminescent immunoassay for the detection of antibodies to *T. cruzi*, on an automated next generation platform, Alinity s.
Methods: The performance of the new automated chemiluminescence immunoassay for the detection of antibodies to *T. cruzi* was evaluated on the Alinity s automated platform and compared to another on-market chemiluminescent immunoassay. Precision was assessed over 20 days using a panel of positive and negative samples. Specificity was evaluated on 5,080 blood donor samples.
Results: Precision was 7% CV or less for positive samples over 20 days. The overall specificity in a blood donor population was 100.00% (5,080/5,080).
Summary/Conclusions: These results indicated the new automated Alinity s anti-*T. cruzi* assay provided very good performance in specificity, which was comparable to the current on-market anti-*T. cruzi* assay and is equally suitable for use in universal screening in endemic and selective donor screening in non-endemic countries.

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SELECTIVE TESTING OF AT-RISK BLOOD DONORS FOR *TRYPANOSOMA CRUZI* AND PLASMODIUM SPP. IN ITALY

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Background: Population migrations and overseas travels for tourism or work to regions at risk for tropical diseases are increasing, and represents potential transfusion-transmitted infections in non-endemic disease countries. The Plasmodium spp. and the protozoans Trypanosoma cruzi, the causative organisms of malaria and Chagas Disease (CD), are becoming a major focus in the blood transfusion community. Italian National guidelines of the Blood Transfusion Service has been recently revised, requiring a deferral period of 6 months for prospective blood donors at risk for malaria or CD and, differently from previous guidelines, included a mandatory selective serological testing before admission to blood donation.
Aims: Aim of the work was the application of the new Italian National guidelines for the screening of at-risk blood donors for malaria and CD.
Methods: According to National guidelines Plasmodium spp. antibodies testing was applied to donors who were born or stayed longer than 6 month in endemic area, who had positive malaria anamnesis, or who have recently travelled in endemic areas (after a 6-months deferral period). These donors were screened with an EIA malaria test for Plasmodium spp. (Bio-Rad, Cressier, Switzerland), detecting antibodies at all stages of infection and against all four species known to cause human malaria (P. falciparum, P. vivax, P. ovale and P. malariae). The specificity of the assays was 92.8%. Anti-Trypanosoma Cruzi Ab testing was applied to donors directly originating or whose mothers originated from endemic areas, or who have travelled in a CD-endemic country. These donors were tested with the Chagas CMIA test system (Abbott, Delkenheim, Germany); the specificity of the assay was 99.94%.
Results: During the first 8 months (July 2016–Feb 2017) of the application of the new guidelines in the North of Italy, 3,849 blood donors at risk for malaria were selected for Plasmodium spp. Ab screening and 181 were confirmed positive. The vast majority of these donors (90%) originating from an endemic country (mainly sub-saharan Africa). Only 17 donors had an European origin and had travelled in endemic areas. The distribution of anti-Plasmodium spp. Ab among these subjects was homogeneous along the entire range of positivity. This reflects the presence of semi-immune subjects, former residents of endemic areas, which are the subjects at higher risk of transmitting malaria trough blood transfusion, since they are people with potential paste episodes of malaria. Instead low-risk short-term travelling donors resulted almost malaria antibodies negative, with a low impact on donor loss. 2,958 donors at risk for CD were tested for anti-Trypanosoma Cruzi Ab and 6 of them were confirmed positive. All of them originated from South/Central America.
Summary/Conclusions: Malaria and CD in non-endemic countries may represent a certain risk for blood transfusion. In particular, the proportion of imported malaria cases due to immigrants has increased during the last decades, with higher rates associated with settled immigrants. The identification of Plasmodium spp and Trypanosoma cruzi antibodies is essential in these subjects. Italy is now dealing with a selective testing approach and the preliminary data here showed suggested that it is the most appropriated way to avoid the risk of transfusion-transmission of these diseases.

Newly emerging pathogens and other transfusion related pathogens

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VIRAL METAGENOMIC ANALYSIS OF BLOOD DONATIONS WITH POSITIVE MARKERS (HBV+, HCV+, HIV+)

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Background: The risk of transmission of a viral infection constitutes one of the major risks for the blood bank activity. Viral threats corresponding to pathogens HBV, HCV, and HIV are well defined; they have been associated, however, to the progressive identification of new viruses, or divergent variants, in human blood. Based on related host immune disorders, risk and intensity of opportunistic infections in individuals positive for HBV, HCV or HIV are increased; consequently, a better knowledge of potentially associated co-infections should contribute to keep a high level of blood transfusion safety.
Aims: In order to gain new insights about the diversity of viruses potentially present in blood products, we explored the virome content of blood donations excluded from transfusion protocol (HBV DNA-, HCV RNA-, and HIV RNA-positive samples) using massive sequencing protocols and bioinformatics analysis.
Methods: 150 plasma samples obtained from the French Blood Agency National Plasma Bank (Tours, France) were studied. One-ml plasma aliquots were clarified by centrifugation, filtered, and subsequently treated with nucleases. Particle-protected DNA/RNA templates were recovered, converted into double-stranded DNA using combined reverse transcription/2-h Phi29 DNA polymerase treatments, and subsequently used for the preparation of next-generation libraries and their analysis (MiSeq, Illumina). Resulting paired-end reads (2×300 nt) were analysed using bioinformatics workflows including quality trimming and filtering steps, human and bacterial sequences removal, *de novo* assembly, and final taxonomic assignations. Additional molecular protocols (sequence extensions, cloning,...) were also applied.
Results: Bioinformatics treatment of reads and subsequent molecular analysis of partial/complete sequences characterized allowed the identification of diverse viral families, including *Anelloviridae*, *Flaviviridae*, *Genomoviridae*, *Hepadnaviridae*, *Papillomaviridae* and *Retroviridae*. The distribution of associated viral isolates was investigated for each plasma sample tested.
Sixteen plasma samples (6 HCV+ and 10 HIV+) exhibited pegivirus sequences (HPgV/GBV-C, family *Flaviviridae*). Contigs analysis and subsequent sequences extensions allowed the characterization of nearly full-length genomes, or large representative portions, and their phylogenetic analysis: 15 isolates were assigned to genotype 2 and 1 isolate to genotype 1. Up to 16% genetic diversity was observed between isolates characterized (E2/envelope). Of note, only one HPgV complete genome was described in France up to now (1998).
One plasma (HIV+) was the source of several gemycircularvirus sequences (family *Genomoviridae*); a complete genome (GemyC1c, 2109 nt) was deduced. GemyC1c capsid protein exhibited ~30% pairwise identity with viral sequences identified previously in humans.
Sequences assigned to family *Papillomaviridae*, showing genetic divergences with already described cutaneous HPVs, were retrieved in two samples (1 HCV+ and 1 HIV+).
Several sequences exhibiting very low similarity scores were also identified and are under investigation.
Summary/Conclusions: Data collected underline the interest of viral metagenomic approaches targeting blood donations with positive markers in a view to explore potential co-infections and associated viral diversity.
Details of the study and recent developments will be exposed.

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DETECTION OF ZIKA VIRUS RNA IN PUERTO RICO DONATIONS USING COBAS® ZIKA ON THE COBAS® 6800/8800 SYSTEMS

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Background: In February 2016, the U.S. FDA recommended that all blood donations in areas with active Zika virus (ZIKV) transmission be tested for ZIKV by nucleic acid test (NAT) or treated by pathogen reduction techniques. Screening of blood donations in Puerto Rico began on April 3, 2016 using the **cobas**® Zika test for use on the **cobas**® 6800/8800 Systems.

Aims: To describe the detection of ZIKV RNA in blood donations in Puerto Rico, a ZIKV active area.

Methods: Donations were screened with **cobas**® Zika by individual donation testing. Initial reactive (IR) results were repeated in duplicate. Additional testing included an alternative NAT (AltNAT) assay which is less sensitive than **cobas**® Zika and serology testing for anti-Zika IgM and IgG. Reactive donors were invited to enroll in follow-up, which included **cobas**® Zika and serology testing. A donor was considered to be Zika infected if at least one replicate of repeat testing by **cobas**® Zika was reactive on the index donation or a follow-up sample, reactive by AltNAT on the index donation, or positive for anti-Zika IgM on index or follow-up. All IR donations were also retested at a 1:6 dilution to simulate minipool testing.

Results: A total of 35,543 donations from April 3 – October 9, 2016 were tested with **cobas**® Zika. Of 316 IR donations, 238 were repeatedly reactive (RR), 62 were non-RR, and 16 donations had no repeat testing.

199/238 RR index donations were reactive by AltNAT; 10 of these were IgM positive. Of the 189 IgM negative donors, 76 enrolled in follow-up and 68 were IgM positive. 33/238 RR index donations were negative, equivocal or not tested by AltNAT. 11/33 were IgM positive. Of the 22 IgM negative donors, 11 enrolled in follow-up and 8 were IgM positive.

Of the 316 IR index donations 62 were non-RR; 2/62 were AltNAT positive and IgM positive on index (1) or follow-up (1). Of the 60 donations that were non-RR and AltNAT negative, 47 were IgM positive on index. Of the 13 IgM negative donors, 7 enrolled in follow-up and 5 became IgM positive. 16/316 IR donations had no repeat testing; however 11/16 index donations tested AltNAT positive and 9/16 were IgM positive on index or follow-up samples.

Altogether, of the 316 IR donations, 303 met criteria for true positive on the index donation. Of the remaining 13 donors, 5 were IgM positive on follow-up, 2 were IgM negative, and 6 had no follow-up. 231/316 (73%) IR donations were reactive when retested in a simulated minipool.

Summary/Conclusions: 0.87% of total donations met the criteria as true ZIKV positive. Donor screening with **cobas**® Zika successfully interdicted potentially infectious donations. Only 73% of reactive donations would have been detected in a minipool of 6.

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Cobas® Zika is not commercially available for blood screening use.

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PERFORMANCE AND EVALUATION OF PROCLEIX® ZIKA VIRUS ASSAY ON THE FULLY AUTOMATED PROCLEIX PANTHER SYSTEM

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Background: Zika virus (ZIKV) is a mosquito-borne flavivirus. In most cases, ZIKV infection causes no or mild symptoms but the virus has been determined to cause Congenital Zika Syndrome in infants born to infected mothers and it has been associated with sometimes fatal cases of Guillain-Barré syndrome in infected adults. Although the primary route of infection is through the bite of a mosquito, sexual transmission, transmission from mother to fetus, and transfusion transmission of ZIKV have been reported. To facilitate fully automated testing for ZIKV RNA, a transcription-mediated amplification (TMA) assay for the qualitative detection of ZIKV RNA on the Procleix Panther System was developed and evaluated. The Procleix Zika Virus Assay uses the same technology as the Procleix nucleic acid tests, consisting of lysis and target capture of viral RNA followed by TMA and chemiluminescent detection. To mitigate the risk of false negative results, the assay targets 2 separate regions of the ZIKV genome and includes an internal control to validate each reaction.

Aims: As part of the assay qualification prior to its use for donation screening at the American Red Cross under an Investigational New Drug (IND) protocol, the preliminary performance characteristics of the investigational Procleix Zika Virus Assay on the Procleix Panther System were evaluated.

Methods: Analytical sensitivity was determined by probit analysis of results from testing 40–144 replicates per level of serially diluted *in vitro* synthesized RNA transcript and a ZIKV positive plasma specimen that was quantified by real-time PCR and digital PCR. Specificity was assessed by testing plasma specimens from 9,400 unlinked normal U.S. donors by the American Red Cross (Gaithersburg, MD). Cross reactivity to other blood borne pathogens (HIV, HCV, HBV, West Nile virus, and Dengue virus) was also evaluated.

Results: Based on probit analysis, the Procleix Zika Virus Assay showed 95% detection at 11.7 copies/ml (95% CI: 9.8–14.6) and 50% detection at 2.9 copies/ml (95% CI: 2.5–3.4) for *in vitro* synthesized RNA transcript (basing on the sequence of isolate MR766, AY632535), and 95% detection at 3.9 copies/ml (95% CI: 3.2–4.8) and 50% detection at 1.1 copies/ml (95% CI: 0.9–1.2) for ZIKV positive plasma (from Brazilian Zika positive blood donor). Assay specificity was evaluated using two reagent lots (6,400 donations for lot 1 and 3,000 donations for lot 2). One false reactive result was observed. The specificity of the assay was 99.99% (95% CI: 99.94–100.00). No cross reactivity was observed when samples from 10 HIV-positive donations, 10 HBV-positive donations, 10 HBV-positive donations, 100 West Nile virus-positive donations, and 120 Dengue virus positive donations were tested.

Summary/Conclusions: The investigational Procleix Zika Virus Assay demonstrated high sensitivity and specificity. No cross reactivity was observed with major blood borne pathogens. Currently, the Procleix Zika Virus Assay is used to screen individual donation under IND protocol in U.S. and is CE-marked for use in countries that recognize CE-mark.

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ZIKA VIRUS AND SAFETY OF SUBSTANCES OF HUMAN ORIGIN – A GUIDE FOR PREPAREDNESS ACTIVITIES IN EUROPE

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Background: The Zika virus epidemic and a possibility of autochthonous transmission of Zika virus in Europe pose a threat to the safety of the substances of human origin (SoHO) because asymptomatic infected residents of areas with a local transmission, as well as travellers returning from affected areas, may donate SoHO infected with Zika virus in Europe. The risk of transmission of Zika virus by transfusion, transplantation or assisted reproduction technologies has not been sufficiently quantified yet, but cannot be ignored. While Zika virus infection is considered a mild disease for the general population, the severity of foetal impairment indicates the need to reduce the risk of infection especially in pregnancy and women of childbearing age.

Aims: We present a current guide for preparedness activities on the safety of SoHO in Europe during the outbreaks of Zika virus infection. The guide has been developed to support the operational preparation and implementation of national preparedness plans in EU/EEAM Member States.

Methods: The SoHO team at the European Commission's Directorate-General Health and Food Safety established a multi-country working group of 17 experts from the blood, tissues and cells, and organs sectors in March 2016 to support European Centre for Disease Prevention and Control (ECDC) in the preparation of the guide. The guide is based on a rigorous evaluation of the evidence base, current EU legislation, risk assessment and all related recommendations. Addressing an epidemic of Zika virus disease required a further consultation with the public health, animal health and entomological expertise, National Competent Authorities (NCA) for SoHO, and the vigilance services. This multidisciplinary approach allows for continuous risk assessments to facilitate appropriate and timely decision-making in transfusion and transplantation medicine. The guide was published in August 2016 and updated in spring 2017.

Results: Key elements of a guide cover affected areas, risk assessment, safety measures, SoHO supply and communication among all parties. For each of these elements, the guide proposes activities that can be undertaken at the EU, national and local levels. The guide introduces a simplified scheme of affected countries and territories. Risk assessment and safety measures are defined separately for blood, cells and tissues, and organs. It also defines triggers for the implementation/discontinuing the measures and anticipates a reassessment of the measures according to the local conditions in particular countries. Special attention has been applied to screening and laboratory capacities in the EU, a collaboration between countries and strategies to maintain SoHO supply within the country. Communication strategy addresses all levels and relevant stakeholders to assure the consistency of information across Europe. NCAs for SoHO use a web-based rapid alert system for blood (RAB) and a rapid alert system for tissues and cells (RATC) to exchange essential information between the Member States. RAB/RATC are used in parallel with national vigilance systems and establishments for SoHO, which collect and manage alerts on SoHO donated and used in the Member States.
Summary/Conclusions: The guide sets out a common frame which may help EU countries to develop national preparedness plans to prevent Zika virus transmission through SoHO.

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NUCLEIC ACID TESTING OF BLOOD DONORS FOR ZIKA VIRUS

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Background: In countries experiencing outbreaks of arboviruses, asymptomatic carriers may candidate as blood donors, ultimately leading the transfusional transmission (TT) of the agent a plausible possibility. In a French Polynesia study, 3% of donors were positive and only 0.7% reported symptoms. As the clinical outcome of TT Zika is unpredictable, NAT screening has been proposed to mitigate the risk.
Aims: The aim of this study was to develop, validate and implement an "in-house" method to detect Zika RNA in blood donations, further incorporating it into the routine.
Methods: Primary plasma tubes from donors providing an informed consent are submitted to nucleic acid extraction using magnetic particles chemistry technology on automated platform. After extraction, NAT set-up is performed in the robotic pipettor, where an amplification mixture containing primers and probes for Zika and an internal control in duplex are added. Real-Time polymerase chain reaction is then performed. One run consists of up to 70 donations and the whole process takes from 4–6 h. A commercially available quantitated Zika RNA was used to estimate the assay limit of detection and determine the viral load on a cell culture supernatant (CCS).
Results: A negative plasma spiked with Zika virus from CCS at a final concentration of 13 copies/ml is used as a running control. From May 23rd 2016 to July 28th 2017, 2,600 samples were tested; 5 (1.1%) were considered invalid due to internal control failure and were repeated and released on the next day. Standard deviations of Ct values from Zika positive control (N = 28, Mean Ct = 30.8; SD Ct = 0.94) and the internal control (N = 471, Mean Ct = 28.0; SD Ct = 1.37) are low, reflecting the reproducibility of the method. No Zika RNA reactive sample was identified.
Summary/Conclusions: It was possible to determine the laboratory characteristics of the developed method and the test showed feasible to be incorporated to the blood screening routine. Studies are still necessary to identify the real risk of ZIKA TT disease. This method represents an alternative to warrant the safety of the blood supply against the emerging Zika virus epidemics. Until now, none of Zika RNA+ donation was detected in our screening blood donors routine.

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DEVELOPMENT OF ZIKA VIRUS RNA REFERENCE REAGENTS AND LOT-RELEASE PANEL AS A RESPONSE TO A PUBLIC HEALTH EMERGENCY

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Background: Since its first reported appearance in the Western Hemisphere in 2015, Zika virus (ZIKV) has spread through the Americas. ZIKV infection has been associated with severe neurological outcomes such as Guillain-Barré syndrome and fetal microcephaly. ZIKV poses a transfusion-transmission risk because viremia appears before symptoms develop, and approximately 80% of infections never produce symptoms. Four cases of transfusion-transmitted ZIKV from three donors have been reported in Brazil, and rates of viremic blood donors during outbreaks were 2.8% in French Polynesia in 2014 and 1.1% in Puerto Rico in 2016. Nucleic acid testing (NAT) is the most sensitive method to screen blood donors for ZIKV infection. Two ZIKV NAT assays are in use for blood screening under IND, but none are currently FDA-approved.
Aims: The aim of this project was to respond to an emergency need by producing and fully characterizing reference reagents for ZIKV RNA and making them available to assist assay development and evaluation.
Methods: Two strains of ZIKV (PRVABC59, Puerto Rico-2015; and FSS13025, Cambodia-2010) were used to produce cell-culture-grown stocks, heat-inactivated at 56°C for 60 min, and diluted in human plasma (BaseMatrix). Heat-inactivation was confirmed by back-titration. The material was initially pre-characterized in collaboration with five proficient laboratories in the US and further characterized by 21 laboratories worldwide as part of studies to establish the WHO ZIKV International Standard. Study participants tested the reagents for ZIKV-RNA using their NAT assay(s) in serial dilution to determine the end-point, followed by testing half-log dilutions around that end-point to confirm the titer. Estimated units/ml were calculated using Probit analysis. Lyophilized formulations were prepared for both reference reagents. Accelerated degradation studies were conducted to assess performance of the lyophilized reagents after incubation at +4°C, +25°C, +37°C and +45°C, and stability studies are ongoing to assess long-term reagent stability when stored at +4°C, −20°C and −80°C.
Results: The ZIKV Reference Reagents had an estimated overall mean of 6.04 log10 units/ml for PRVABC59 and 5.59 log10 units/ml for FSS13025. Real-time stability studies are ongoing; preliminary results show good stability at −20°C for both reagents, and better stability for the FSS13025 formulation, with no deterioration also at +4°C and −80°C. Accelerated degradation studies showed degradation starting after 1 month at +37°C and +45°C. A lot-release panel ranging from 200 – 0 copies/ml has been produced using the reference reagent materials. The panel is currently being assessed in collaborative studies as done previously. Additionally, we have performed complete genomic sequencing including the 5′ and 3′ non-coding regions for strains PRVABC59 and FSS13025 and uploaded these to Genbank.
Summary/Conclusions: We have produced ZIKV RNA Reference Reagents to facilitate evaluation of existing NAT assays and development of novel ZIKV assays. The CBER ZIKV RNA Reference Reagents are available in both lyophilized and liquid-frozen formats for use to assist assay development and establish and evaluate assay performance in response to the ZIKV public health emergency.

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A QUANTITATIVE RISK ASSESSMENT MODEL FOR TRANSFUSION TRANSMISSION OF ZIKA VIRUS IN PUERTO RICO

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Background: Emergence of Zika Virus in Puerto Rico raises concerns for transfusion-transmission of Zika virus (TTZIKV) and associated microcephaly in congenitally exposed infants.
Aims: FDA developed a quantitative risk assessment model to estimate the residual risk of TTZIKV from the red blood cells (RBC) collected in the Pureto Rico that have been tested with an investigational individual donor nucleic acid test (ID-NAT).
Methods: The model estimated the TTZIKV risk from infected, asymptomatic donors. Residual risk from false negative test errors was calculated. Predictive coefficients were derived from model simulations and used to predict the risk outcomes directly from either the rate of reported clinical cases or the NAT positive rate in donors.
Results: Estimates for the numbers of infectious RBC units per 100,000 donations, monthly infectious RBC units, TTZIKV cases in pregnant women and immunocompromised recipients were generated using predictive coefficients. Based on a report of 33,227 clinical ZIKV cases for the period April 3rd-November 17th, the model predicted a mean of 153 cumulative cases (less than 1 pregnant women) with testing; while 1,128 cases (5 in pregnant women) should ID-NAT is not implemented. An ID-NAT donor testing with a 0.5-day window period and 99.8% clinical test sensitivity was predicted to reduce the TTZIKV risk by a mean of about 86%.
Summary/Conclusions: Model validation indicated that the model reliably predicted donor risk. This modeling approach can be applied to other regions with active ZIKV transmission.

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PRESENCE OF ANTI-ZIKV IGM IN ZIKV RNA POSITIVE SAMPLES AMONG BLOOD DONORS FROM AN AREA WITH ACTIVE VIRAL CIRCULATION, BRAZIL

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Background: Brazil has experienced one of the largest Zika virus (ZIKV) epidemic among the Latin American countries. The introduction of ZIKV in 2015 and the rapid spread throughout the country led the health care to pay attention to the transfusion transmission (TT) risk of this infection. Apart from this, the prevalence of the acute infection among Brazilian blood donors is completely unknown.
Aims: The goal of this study is to evaluate the prevalence of anti-ZIKV IgM in ZIKV RNA positive samples from volunteer blood donors from the Northeast part of the São Paulo State, Brazil.
Methods: Plasma samples were tested for anti-ZIKV IgM using the *ZIKV ELISA for qualitative determination of IgM antibodies to Zika virus* in human serum and plasma kit (DIA.PRO).
Results: From 37 samples positive for ZIKV-RNA, we detected five blood donor samples where the detection of viral RNA was accompanied by the presence of anti-ZIKV IgM (13.5%).
Summary/Conclusions: It is currently unknown if the presence of antibodies concomitantly with viral RNA can influence the transmission by blood transfusion. For that purpose, studies which involve inoculation of positive for ZIKV samples with presence of antibodies in appropriate animal model must be performed in order to examine the clinical course of the infection.
This study points out the urgent need for discriminatory and/or routine ZIKV-NAT diagnosis in order to understand the ZIKV biological and molecular characteristics in blood donors such as viral load, ZIKV persistence in blood derivatives and the use of pathogen reduction strategies to prevent TT-ZIKV.

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Abstract has been withdrawn.

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ROBUST INACTIVATION OF DUCK HEPATITIS B VIRUS WITH AMUSTALINE/GSH IN WHOLE BLOOD

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Background: Duck Hepatitis B virus (DHBV) is a small, enveloped, dsDNA virus of the *Avihepadnavirus* genus, a group of viruses in the family *Hepadnaviridae*. DHBV can be utilized as a model virus for human hepatitis B virus (HBV). Human hepatitis B is a potentially life-threatening liver infection caused by HBV that can result in chronic infection and an increased risk of death from cirrhosis and liver cancer. Hepatitis B prevalence is highest in sub-Saharan Africa and East Asia, where between 5–10% of the adult population is chronically infected (WHO). In highly endemic areas, hepatitis B is most commonly spread through horizontal transmission via exposure to infected bodily fluids, highlighting the risk associated with blood transfusion. The inability to consistently supply blood components makes WB transfusion common, and since transfusion-transmitted diseases are prevalent in the developing world, the development of a robust WB pathogen inactivation system is desirable. The INTERCEPT Blood System for WB uses the small molecule amustaline to form covalent adducts and crosslinks within nucleic acids of leukocytes and contaminating pathogens to prevent replication. The process includes addition of 0.2 mM amustaline and 2 mM glutathione (GSH) and 24 h incubation at room temperature (RT). At the conclusion of the RT incubation, the treated WB unit is suitable for storage up to 7 days.
Aims: The objective of this study was to evaluate the inactivation of DHBV with the INTERCEPT™ Blood System for Whole Blood (WB) to support the Swiss Red Cross Humanitarian Foundation for Whole Blood Pathogen Inactivation for Africa.
Methods: For each experiment, a single WB unit was spiked with DHBV to a final concentration of ~$10^{4.5}$ $TCID_{50}$/ml and treated with amustaline and GSH. A control sample was removed prior to the addition of amustaline, serially diluted up to 100,000-fold and inoculated onto duck hepatocytes to determine the pre-treatment titer, resulting in a control titer of 4.6 $\log_{10}$ $TCID_{50}$/ml. Each unit was then dosed with amustaline and a test sample was removed after 24 h to determine the levels of inactivation. Test samples were diluted 1:2 to 1:10 and inoculated onto duck hepatocytes. The Limit of detection (LOD) was determined to be <-0.7 $\log_{10}$ $TCID_{50}$/ml. The plates were incubated for 10 days at 37°C, fixed with ethanol and the presence of viable DHBV determined by indirect IFA with a mAb to the DHBV envelope protein. Log reduction was calculated as the difference between the mean titer in pre-amustaline samples and the mean titer in the 24 h post-amustaline samples.
Results: Robust inactivation of DHBV in WB was achieved to the LOD, at >5.3 $\log_{10}$ (n = 4). This corroborates previous results achieved in AS-5 red blood cells with 0.2 mM amustaline and 20 mM glutathione, resulting in inactivation to the LOD at >5.1 $\log_{10}$ of DHBV (n = 4).
Summary/Conclusions: Duck hepatitis B virus was inactivated to the limit of detection in WB after treatment with amustaline and GSH using the duck hepatocyte infectivity model.
The INTERCEPT Blood system for WB or RBCs is not approved for use.

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A STUDY OF WEST NILE VIRUS INCIDENCE AMONG BLOOD DONORS IN SLOVENIA

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Background: West Nile Virus (WNV) can cause West Nile fever infection, which can further develop into meningitis and encephalitis. Virus is transmitted to humans and animals via the bites of infected mosquitoes. The natural host of the virus are birds. Infection outbreaks in human are typically occurring in periods with the highest mosquito activity, i.e. between July and September. Virus can be also transmitted to humans by organ transplants and blood transfusions. Therefore, it presents a potential threat to the safety of blood supply. As most infections in humans are asymptomatic, the risk of transmission by transfusion is high. Up to now, in Slovenia, blood safety is ensured by deferral of blood donors who lived or traveled in countries with WNV outbreaks 28 days before donation. In Slovenia, there was only one confirmed case of WNV infection in a patient in 2013. However, outbreaks of WNV infections are quite frequent in our neighboring countries, Italy and Croatia, in areas that are close to Slovenia.
Aims: The aim of our retrospective study was to determine the incidence of acute WNV among regional groups of blood donors in Slovenia. For this purpose, we screened samples of blood units donated within the period between July 29 and September 11, 2015. The results of the study will help us to evaluate the risk for potential transfusion transmission of WNV and to define measures of prevention strategy.
Methods: Donors' samples tested were grouped into five geographic regions according to the sites of blood donation and residency. Plasma samples of selected individuals were taken from the seroteque at Blood Transfusion Centre of Slovenia and tested individually on Procleix Panther System (Grifols) with Procleix WNV assay (Grifols) using Transcription-Mediated Amplification (TMA) technology. Additionally, internal quality control (IQC) samples (QConnect WNV RNA Control, NRL, Australia) were tested in every sample batch.
Results: 3,888 tested samples, representing 5% of all donations in Slovenia in 2015, were reported valid and non reactive. Test results were validated with IQC samples.
Summary/Conclusions: Results show zero incidence of WNV infection among donors in the peak of possible WNV outbreak period in year 2015, resulting in a very low risk for transfusion transmitted WNV infection in Slovenia. However, migrations of Slovenian residents to neighboring or remote countries with constant occurrence of WNV cases have to be considered when examining the donor prior to donation and deferral of such donors is in place. Future surveillance of epidemic situation in surrounding areas is of crucial importance for preparedness for quick response and potential emergent implementation of WNV NAT screening scenario in case of WNV outbreak in Slovenia.

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ENHANCING THE EXPRESSION OF CCR5 RECEPTOR BY TAX-1 GENE OF HTLV-1

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Background: Tax-1 is produced by Human T-cell Leukemia Virus type 1 and serves as a key transcriptional regulatory gene product. Tax-1 plays a crucial role in transactivating genes of infected cells by employing their transcriptional factors. This modulation includes induction of genes which encode expression of CC-chemokines and their receptors.
Aims: In this study, a recombinant vector containing Tax-1 gene was made and was tested for its ability to induce CCR5 (CC-chemokine receptor) expression in K562 cell line.
Methods: In order to conduct this research, HTLV-1 positive blood samples were obtained from Urmia blood transfusion center. After DNA extraction, a complete sequence of Tax-1 gene was amplified by specific primers. Recombinant vectors carrying Tax-1 gene were synthesized and transformed into E. coli. After bacteria transformation, bacteria containing recombinant plasmid were selected and purified. Then the recombinant shuttle vectors, pCDNA3.1-TAX, was transfected to the cell culture (K562 cell line). Expression of CCR5 was measured after 72 h by Real Time PCR method compared to control cell culture.
Results: Cloning of Tax-1 gene in the vector, pCDNA3.1, was confirmed by sequencing method. Expression of Tax-1 gene was confirmed by Real Time PCR and also, expression of CCR5 gene showed a 15-fold increase in compared to mock-treated controls (GAPDH gene) ($P < 0.05$).
Summary/Conclusions: The results of this study showed that Tax-1 protein could increase levels of CCR5 expression which is an important co-receptor for HIV-1 entry into the cells, especially in endemic areas.

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PERFORMANCE OF A NEW AUTOMATED ASSAY FOR ANTIBODIES TO CMV ON THE ALINITY S SYSTEM

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Background: Infections with Cytomegalovirus (CMV), a member of the herpesvirus family, are common in man and are usually mild and asymptomatic. However, in pregnant women, newborns, and immunocompromised individuals, CMV infections may pose a significant medical risk and many countries reserve CMV negative blood units for donors at risk. Blood centers require very high throughput anti-CMV IgG assays with high specificity and sensitivity to maintain the safety of the blood supply. In addition, continued pressures on laboratory operations demand that assays perform on platforms capable of increased walk away time and enhanced automation in areas of reagent management, retest options, and commodity/waste management.
Aims: To evaluate the overall performance of a new chemiluminescent immunoassay for the detection of IgG antibodies to CMV, on the automated Alinity s system.
Methods: The performance of the new automated chemiluminescence immunoassay for the detection of antibodies to CMV was evaluated on the Alinity s automated platform and compared to another on-market chemiluminescent immunoassay. Specificity/sensitivity as measured by % agreement was evaluated on 4,654 blood donor and 200 hospital/diagnostic samples. Precision was determined over a 17 day study.
Results: The overall % agreement in a blood donor population was 99.10%. In the precision study the % CV was less than 9%.
Summary/Conclusions: These results indicated the new automated Alinity s CMV IgG assay provided acceptable performance in specificity/sensitivity and precision, which was comparable to the current on-market CMV IgG assay.

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THE COMPARATIVE STUDY OF CYTOMEGALOVIRUS IGG SEROPREVALENCE AMONG BLOOD DONORS IN WUHAN IN 2015 AND 1990

H Bi

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Background: Human cytomegalovirus (HCMV) can be transmitted by blood transfusion. The recommended way to prevent transfusion-transmitted CMV (TT-CMV) is providing the HCMV negative blood from donors by serologic screening, which is proved effective in Europe and America, but it has not been adopted in China, yet. Whether the strategy is suitable in Wuhan city in China or not, the current seroprevalence of HCMV in donors in Wuhan should be investigated. As early as 1990, we analyzed seroprevalence of HCMV in blood donors in Wuhan and 20 years later, we investigated it again in 2015.
Aims: By the comparison of the HCMV-IgG seroprevalence among blood donors in Wuhan in 2015 and 1990, to find the cause of the change of HCMV seroprevalence in the recent 20 years, thus to benefit establishing the effective strategy for preventing CMV infection.
Methods: HCMV-IgG in 440 plasma samples from blood donors in Wuhan in 2015 were tested and the seroprevalence were compared with the one in 1990.
Results: In 2015, the HCMV-IgG seroprevalence among blood donors in Wuhan was 88.24%, and the one of male was 88.24% and female was 89.60%, which were all lower than the one in 1990 (total was 95.20%, male was 95.43%, female was 94.95%).

Table 1 HCMV-IgG seroprevalence among blood donors in Wuhan in 2015

Age	Male Positive/test	Positive rate (%)	95% CI	Female Positive/test	Positive (%)	95% CI	Total Positive/test	Positive rare (%)	95% CI
18–23	84/101	83.17	75.75–90.59	70/82	85.36	77.55–93.18	154/183	84.15	78.81–89.49
24–29	72/80	90.00	83.28–96.72	59/67	88.06	80.09–96.03	131/147	89.11	84.02–94.21
30–39	27/29	93.10	83.29–102.91	24/25	96.00	87.74–104.26	51/54	94.44	88.13–100.76
40–49	19/20	95.00	84.53–105.47	22/22	100.00		41/42	97.62	92.81–102.43
50–55	8/8	100.00		6/6	100.00		14/14	100.00	
Total	210/238	88.24	84.11–92.36	181/202	89.60	85.36–93.85	391/440	88.86	85.91–91.81

Table 2 HCMV-IgG seroprevalence among blood donors in Wuhan in 1990

Age	Male Positive/test	Positive rate (%)	95% CI	Female Positive/test	Positive rate (%)	95% CI	Total Positive/test	Positive rate (%)	95% CI
18–23	26/31	83.87	70.16–97.58	47/49	95.92	90.18–101.66	73/80	91.25	84.92–97.58
24–29	37/39	94.87	87.63–102.12	20/23	86.96	72.00–101.85	57/62	91.94	84.96–98.91
30–39	94/96	97.92	95.01–100.83	67/71	94.37	88.87–99.86	161/167	96.41	93.56–99.26
40–49	47/48	97.92	93.73–102.11	50/51	98.04	94.10–101.98	97/99	97.98	95.16–100.80
50–55	5/5	100.00		4/4	100		9/9	100.00	
Total	209/219	95.43	92.65–98.22	188/198	94.95	91.87–98.03	397/417	95.20	93.14–97.26

Summary/Conclusions: The CMV prevalence among blood donors in Wuhan has fallen, which is related to the improvement of the social and economic status of our country and the only-child policy. With the advent of the two-child policy and Chinese aging, the controlling of CMV infection should be strengthened.

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Abstract has been withdrawn.

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HCV AND HBV IN YOUNG BLOOD DONORS

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Background: In Italy, in recent decades, there has been a profound change in the epidemiology of viral hepatitis B and C, for the contribution of different causes In particular: improved hygiene and socio-economic conditions; greater knowledge and awareness of the risk of transmission, the introduction of important preventive measures such as screening of blood donors; the adoption of universal precautions in health care. Hepatitis HBV, such as other blood-borne hepatitis C which showed a significant and steady reduction in the incidence in recent decades. Today those who develop hepatitis B and hepatitis C are mostly males between 35 and 54 years of age.
Aims: The purpose of the study was to evaluate the progress of HBV and HCV infections in young Italian blood donors included in the range 18–35 years afferent to the Aversa Transfusion Center, that it is located in areas where the prevalence of these infections is still high.
Methods: Were screened 18,796 young donors in the period 2014–2016. The tests were performed at the Sit ASL Ce, with chemiluminescence method, performed with Architect i2000 by Abbott. The search for the both virus (B;C) in molecular biology, was performed at the Nat reference center of Caserta. with the Cobas by Roche.
Results: Of 18,796 donors 7,863 belonged to the 18–25 range and 10,933 to the 26–35 range. The total number of young positive donors to HCV and HBV infections can be described as follows:
HCV/HBV 18–25 range: 6 (0.07%).
HCV/HBV 26–35 range: 34 (0.3%).
Summary/Conclusions: from the data obtained it is known that the prevalence of HCV and HBV infection mostly affects the group between 26–35 years, while it is evident a decrease of the same in the 18–25 range according to national statistics. It is therefore important to implement the information and prevention policies by the transfusion center and associations (Avis) with days dedicated to information, in schools or meeting places in order to reach a 'further reduction of infections.

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HCMV-IGG POSITIVE RATE IN BLOOD DONORS IN CHINA: A META ANALYSIS

H Bi

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Background: Human cytomegalovirus (HCMV) can be transmitted by blood transfusion. The recommended way to prevent transfusion-transmitted CMV (TT-CMV) is providing the HCMV negative blood from donors by serologic screening, which is proved effective in Europe and America. Due to unclear data of HCMV epidemiology in blood donors and clinical demand of HCMV negative blood in China, serologic screening of HCMV in donated blood has not been adopted in China, yet.
Aims: To investigate seroprevalence of HCMV in Chinese blood donor population, which will provide the basis for the establishment of procedure and policy to prevent TT-CMV in China.
Methods: We electronically searched databases including PubMed, EMbase, CNKI, VIP, WanFang Data and CBM to collect studies about seroprevalence of HCMV in Chinese blood donor population. Two reviewers independently screened literature, extracted data, and assessed the methodological quality of included studies. Then, meta-analysis was performed using R software (R3.1.1).
Results: A total of 20 studies were included, containing 25,918 tests, among which 21,971 tests are positive. Heterogeneity exists among the different cities, I^2 >50%, $P < 0.1$. The combined HCMV-IgG positive rate in Chinese donors was 87.1% with 95% CI 79.4% to 93.2%. HCMV-IgG positive rate among male was 86.6% with 95% CI 78.6% to 93.0%, while among female was 87.9% with 95% CI 78.6% to 94.9%, and there was significant difference between male and female ($P < 0.01$). HCMV-IgG positive rate among 12–25 age group was 85.3% with 95% CI 81.9 % to 88.3 %, while among 26–60 age group was 91.7% with 95% CI 87.9% to 94.9%, and there was significant difference between both groups ($P < 0.01$). HCMV-IgG positive rate among male in 18–25 age group was 81.3% with 95% CI 75.9% to 86.1 %, while among female in 18–25 age group was 90.2% with 95% CI 81.2 % to 96.7 %, and there was significant difference between both groups ($P < 0.01$).
Summary/Conclusions: as the prevalence of HCMV in blood donor population is very high in most Chinese cities, the strategy to prevent of TT-CMV by serologic screening in all blood donors for HCMV is not suitable in China; age and gender were the factors for the seroprevalence of HCMV in blood donor population in China.

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HTLV-1 INFECTION IN IRANIAN BLOOD DONORS, AND PATIENTS WITH BETA THALASSEMIA: A PHYLOGENETIC ANALYSIS

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Background: Human T-cell leukemia virus has been associated with different types of disease such as Adult T-cell lymphoma (ATL) and Myelopathy/ Tropical Spastic Paralysis (HAM/TSP). Seven subtypes of virus have been identified. As this virus is widespread in some regions (Japan, Africa, and South of America) of the world, this is endemic in parts of Iran too.
Aims: The aim of this study was identification of HTLV-1 genotype and sequence analysis of LTR gene in blood donors and Beta thalassemia patients.
Methods: In this cross sectional study, Samples from 2000 blood donors and 100 beta-thalassemia patients were screened for anti-HTLV-1 antibody by ELISA method (Diagnostic Bio Probe and Nested PCR was performed for both TAX and LTR regions to confirm ELISA results. Purified PCR products were sequenced and analyzed. Phylogenetic tree were constructed by Mega5 software.
Results: Anti-HTLV-1 antibody among blood donors was 2.2% (44 /2000) The PCR results confirmed one out of 2000 samples (0.05%) in blood donors and 8(8%) in beta thalassemia patients *were HTLV*-I positive. All sequenced samples were matched to HTLV-1 subtype a, subgroup A.
Summary/Conclusions: Phylogenetic analysis shows all sequences are related to endemic clusters of Iran.

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PERFORMANCE OF A NEW AUTOMATED IMMUNOASSAY FOR THE DETECTION OF HTLV I AND HTLV II ON THE ALINITY S SYSTEM

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Background: In endemic countries for HTLV, universal blood screening is necessary to detect the presence of antibodies to human T-lymphotropic virus Type I and/or human T-lymphotropic virus Type II (anti-HTLV I/HTLV II). In non-endemic countries, selective testing may avoid unnecessary temporal deferrals for donors at high risk, such as returning travelers from or donors born in countries with a high HTLV prevalence. Blood centers require very high throughput anti-HTLV I/HTLV II assays with high specificity and sensitivity to prevent unnecessary donor deferrals while

maintaining a safe blood supply. In addition, continued pressures on laboratory operations demand that assays perform on platforms capable of increased walk away time and enhanced automation in areas of reagent management, retest options, and commodity/waste management. In the response for the need for increased specificity for such screening assays, we have evaluated an improved automated assay for the detection of antibodies to HTLV I and HTLV II.

Aims: Performance of the new automated chemiluminescence immunoassay for the detection of antibodies to HTLV I and HTLV II was evaluated on the Alinity s system.

Methods: Precision was assessed over 20 days using HTLV I and HTLV II positive samples. Specificity was evaluated on samples obtained from 5,458 European blood donors and 200 diagnostic samples obtained from the US. Sensitivity was evaluated on 400 preselected HTLV I and HTLV II positive samples. Sensitivity and specificity samples were split across 3 reagent lots during testing.

Results: Precision was less than 7.0% for samples over 20 days. Clinical sensitivity was 100.00% (400/400) on preselected HTLV I and HTLV II positive samples. The specificity was 100.00% (5,458/5,458) on a blood donor population and 100.00% (200/200) on diagnostic samples.

Summary/Conclusions: These results indicate that the new automated prototype HTLV I/II assay provided very good performance in specificity, sensitivity, and precision. Sensitivity and specificity were comparable to the comparator assay.

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DESIGN AND PERFORMANCE OF THE ROCHE DIAGNOSTICS ELECSYS HTLV-I/II® ASSAY

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Background: The human T-cell lympho-tropic virus (HTLV) is a retrovirus and is associated with T cell lymphoma and neurodegenerative diseases. Transmission of this infection via infected blood products poses a risk for blood donation recipients. Blood testing is therefore mandatory in many countries in Europe, Middle East, the America and Japan.

Aims: The Elecsys HTLV-I/II® assay was designed with a unique HTLV antigen combination to provide high sensitivity and specificity for screening of diagnostic and blood donation samples.

Methods: Elecsys HTLV-I/II® assay is a qualitative third-generation double antigen sandwich assay with a total duration of 18 min. It employs recombinant antigens representing immunodominant regions of the envelope protein and the capsid protein from HTLV I and HTLV II. Biotinylated antigens and antigens labelled with a ruthenium complex react with anti-HTLV antibodies and form a sandwich complex binding to streptavidin-coated magnetic particles. This electrochemiluminescence immunoassay is intended for use on **cobas e** immunoassay analyzers.

Results: One focus in the development of the Elecsys HTLV-I/II® assay was set on high early seroconverter sensitivity without impact on the specificity. An oligomeric capsid antigen was designed to improve early seroconversion detection. An animal model system was used to assess seroconversion sensitivity and to demonstrate the benefit of the antigen combination.

Roche Elecsys HTLV-I/II® assay was evaluated in a multicenter evaluation with samples from different geographic origins. Specificity in blood donors (n = 11,575) was 99.95%, sensitivity (n = 1,149) was 100%. Specificity in university hospital samples from high prevalence areas and daily routine low prevalence samples (n = 2,399) was 99.83%. A post launch study confirmed the specificity result with 99.96% (n = 5,070).

Summary/Conclusions: Elecsys HTLV-I/II® assay is a very sensitive HTLV screening assay with an outstanding specificity and is suited to detect and verify HTLV infections of all stage as well as to screen blood donors and diagnostic routine patients.

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EPIDEMIOLOGY OF TORQUE TENO VIRUS IN BLOOD DONORS AND ITS IMPLICATION ON BLOOD SAFETY

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Background: Globally, blood transfusions have become a routine process in the hospitals. Torque teno virus (TTV), a single stranded, non-enveloped DNA virus is highly prevalent among the general population throughout the world. TTV was discovered in 1997 in the serum of a Japanese patient with post-transfusion hepatitis of unknown etiology. TTV was first characterized as blood-borne virus and thus named transfusion-transmitted (TT) group of viruses. 1–12% of blood donors from different parts of the world have been reported of having TTV infection. The knowledge on prevalence of TTV in specific regions is expected to assist in the formulation of for virus transmission control in healthy population.

Aims: To assess the molecular epidemiology of TTV in healthy blood donors and find its relationship with Hepatitis B and Hepatitis C seropositivity.

Methods: This single centre cross sectional study was conducted at the Department of Blood Transfusion Services, Shaheed Zulfiqar Ali Bhutto University, Islamabad, Pakistan from November 2015 to August 2016. A total of 188 voluntary blood donor samples were randomly selected after screening on the Abbott ARCHITECT i2000SR Immunoassay. Out of these 188, 50 were HCV positive, 50 were HBV positive, while the remaining 88 were from healthy donors.

For the detection of TTV antigen, testing was done on the automatic ELISA system using the Human TTV kit (Abbexa abx053944, Abbexa Ltd., Cambridge Science Park, Cambridge, CB4 0FN, UK). All collected samples were tested for alanine aminotransferase (ALT) level measurement. All samples were further processed for DNA isolation carried out by using Sambrook and Russel DNA isolation protocol from serum samples. PCR was done to confirm the TTV-DNA in selected samples.

Results: In the present study, 52 (27.6%) samples were found reactive for TTV as detected by ELISA. The same results were found when samples were tested by PCR but the reactivity ratio was different in the three categories of samples. In the ELISA screening, the prevalence of TTV was 20.0% (10/50) in HCV, 36.0% (18/50) in HBV and 24.0% (24/88) in healthy donors. Samples were run on 96 well PCR thermocycler and checked on 2% agarose gel electrophoresis. The prevalence of TTV was found 24.0% (12/50), 40.0% (20/50) and 22.7% (20/88) in HCV, HBV and healthy donors respectively.

The average ALT level found was 35 IU/L. There was no possible association observed between high or low level of ALT among TTV positive subjects and TTV negative subjects. P-value was calculated using SPSS-20 by 1 sample *t*-test. The results showed both ELISA and PCR of equal significance. P-value of the results found equal to 1.00.

Summary/Conclusions: This research project provided basis for implication of TTV in daily routine screening in transfusion services for the better health of blood recipients and general population. There is a need of legislation and immediate steps to be taken to include TTV screening as routine blood screening in blood banks.

P-400

TRENDS OF TRANSFUSION TRANSMISSIBLE INFECTIOUS DISEASES AMONG PAKISTANI BLOOD DONORS

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Background: The prevalence of Transfusion Transmissible Infectious Diseases (TTID) is increasing in developing countries, and this may threaten the biological safety of donated blood.

Aims: Safe blood is a universal right of each human being, therefore present study was planned to evaluate the dissemination and trend of TTID among the blood donor community of Pakistan.
Methods: This retrospective study was conducted at Department of Transfusion Medicine, Punjab Institute of Cardiology (PIC), Lahore, Punjab, Pakistan, from 1st January 2011 to 31st December 2015. Total 79,774 blood donors were enrolled. Four ml blood (Yellow top −3 ml & Lavender top 01 ml) was collected from each donor, and processed for detection of Hepatitis B Virus (HBV), Hepatitis C Virus (HCV), Human Immunodeficiency Virus (HIV), *Treponema palladium (*TP) and Malarial Parasite (MP) by rapid immunochromatographic (ICT) technique.
Results: Out of total 79,774 blood donors, 91% were male and resting 9% were female. The mean age was 44 ± 10 years. From 79,774 subjects only 4.0% (n = 3,209) donors were found infected with any of infectious disease screened. HBV was observed 0.9% (n = 792/79,774), HCV 1.7% (n = 1,407/79,774), TP 1.1% (n = 912/79,774) MP 0.1% (n = 98/79,774) and HIV 0% (n = 0). Co-infectivity was found 0.36% (n = 288) of study population. HBV& HCV was more frequent 37.5% (n = 108) co-infection and HBV & MP was least 1.3% (n = 4) prevalent among seropositive donors.
Summary/Conclusions: Providentially TTID trends are being decreased among Pakistani blood donors, but globally still we are on peak level. Syphilis is an emerging dilemma. Therefore practicable strategies are hardly needed for its eradication and to ensure the availability of safe and healthy blood.

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WEST NILE VIRUS SCREENING IN PORTUGAL

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Background: The West Nile virus (WNV), is a mosquito-borne flavivirus belonging to the *Flaviviridae* family. It is a lipid enveloped RNA virus, first identified in 1937 in Uganda and found in Africa, Europe, Middle East, North America and West Asia. WNV infection was shown to be transmitted by blood transfusion, during the 2002 epidemic in the USA. Since then, several measures have been introduced to reduce the risk of transmission via blood transfusion/solid organ transplantation.
Aims: In the summer of 2015 a case of WNV infection was detected in the south region of Portugal. This region, Algarve, was declared endemic and consequently several measures were implemented nationally, either a 28 days deferral for blood donors who had spent at least one night in that endemic area, or the screening for WNV RNA in individual donation (blood and solid organ). These measures lasted from first of September to 31 October. The Portuguese Blood and Transplantation Institute took the decision to perform the test, considering the need of blood supply and named the Lisbon Blood and Transplantation Center (CSTLisboa) to be encharged of the whole country WNV screening.
Methods: The Nucleic Acid Tests (NAT) screening were implemented in samples from individual blood donation with the kit cobas WNV (equipment cobas 6800), Roche Diagnostics GmbH. It is a real–time PCR test, highly sensitive for both lineages 1 and 2. The test has a simultaneous detection of WNV RNA and an internal control (IC) that helps ensure result integrity. The limit of detection for lineage 1 is 12,9 copies /ml and for lineage 2 is 6,2 copies/ml. Interpretation of Results.
WNV Reactive – Target signal detected for WNV and IC signal may be or may not be detected; WNV Non-Reactive – No target signal detected for WNV and IC signal detected; Invalid – Target and internal control signal not detected.
Results: A total of 4,264 individual blood donors were tested, 2,429 being from Algarve, 1,022 from Lisbon region, 459 from Coimbra region and 354 from Porto region. Nine samples from solid organ donors from Lisbon and 1 from Coimbra were also tested. The blood components from Algarve that were previously stored were only issued after the results. The overall results were negative.
Summary/Conclusions: WNV infection in Portugal is a rare event but in face of this outbreak, the CSTLisboa was able to implement WNV RNA detection in a very short period and this experience made possible the CSTL be prepared for future similar events.
The implementation of NAT screening WNV RNA allowed the clinical use of 4,264 blood units and 10 solid organs during this period of time, not endangering the level of the national blood supply.

P-402

PREVENTING EMERGING AND CO-CIRCULATING TRANSFUSION-TRANSMITTED ARBOVIRUS INFECTIONS

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Background: The consequence of Zika virus (ZIKV) emergence on blood transfusion safety was suspected in French Polynesia (FP) in 2014, confirmed by the demonstration of ZIKV transfusion–transmitted infections (TTIs) in Brazil in 2016 and recently ZIKV-positive blood donations were identified in continental US. While ZIKV has been the center of attention, other arboviruses posing a threat to the blood supply, including dengue virus (DENV), co-circulate in active ZIKV transmission areas.
Since most arbovirus infections are asymptomatic, medical questionnaire is not effective enough at identifying infected blood donors; licensed nucleic acid testing (NAT) assays are not widely available; pathogen reduction technologies (PRT) are available for platelets and plasma but not yet for red blood cells (RBCs).
Aims: In order to prevent known and emerging arbovirus TTIs, we built on the experience gained during the FP and Brazilian ZIKV crisis and share our perspectives on the best approaches to be deployed during the emergence of new pathogens.
Methods: In FP, all platelets were pathogen reduced (technology was implemented in 2010 to prevent DENV TTIs), plasmas were imported from continental France and a laboratory-developed NAT assay was implemented on week 12 of the outbreak for all blood donations. These strategies influenced the approach adopted at Fundação Pró-Sangue (FPS, Brazil), where a laboratory-developed NAT assay was implemented to screen blood components destined for pregnant women and intra-uterine transfusions. PRT was recently approved in Brazil but has so far not been widely adopted by public blood centers.
Results: In FP, 26 patients were transfused with ZIKV-positive blood products before the implementation of NAT. In Brazil, the official recommendations were to avoid collecting units from endemic areas and ask donors to inform the blood center of any diagnosed ZIKV infection or report development of symptoms compatible with infection within 2 weeks following donation. No autochthonous ZIKV infections were described in São Paulo city but one positive blood donation was identified among 28,426 donations. ZIKV TTIs were identified in Brazil but not in recipients getting units from FPS.
Summary/Conclusions: Even though quickly implemented in FP, NAT was not implemented fast enough to prevent transfusion of ZIKV-positive blood products. In Brazil, as NAT was implemented partially and several months after introduction of ZIKV, the number of transfusion with ZIKV-positive blood product is unknown and the number of confirmed ZIKV TTIs may be vastly underestimated. In both countries, the number of DENV TTIs is unknown because blood donations are not routinely screened for DENV. Therefore there is an urgent need for proactive strategies such as PRT that can be implemented ideally in areas of active arbovirus circulation, or immediately after outbreak onset, even while the etiologic agent has not been identified. In addition, recent data demonstrated that arboviruses can be inactivated in RBCs with a research PRT approach.
We recommend testing the efficacy of PRT to inactivate pathogens with potential to emerge and urge the development and licensing of PRT for RBC. PRT use does not exclude the development of specific NATs.

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PRELIMINARY RESULTS ON THE PREVALENCE OF ACUTE HEPATITIS E VIRUS INFECTION IN CANADIAN BLOOD DONORS

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Background: Hepatitis E virus (HEV) is known to be transfusion- transmissible. The prevalence of acute HEV infection varies greatly from one country to another. As part of our risk assessment for this infection, we carried out a first study in 14,000 Canadian blood donors. In a subset of 4,000 donor samples the seroprevalence was 5.9%. Seropositivity was associated with male sex and increasing age, and post-test

interviews identified having lived in another country and spending time with farm animals as risk factors. However no donor samples were positive for HEV by an in-house nucleic acid test (HEV-NAT) in pools of 100. Since that study suggested exposure to HEV in Canada but used an HEV-NAT with a limit of detection of 250 IU/ml, and since only 14,000 donations were tested, we felt is was appropriate to do a larger study using a more sensitive HEV-NAT method.

Aims: To determine the prevalence of acute HEV infection in 50,000 Canadian blood donors using a highly sensitive HEV-NAT.

Methods: Donors were informed about the study in the pre-donation reading materials. Linked samples from approximately 50,000 Canadian whole blood donors including 30,000 from Canadian Blood Services (CBS) and 20,000 from Héma-Québec (HQ), are being collected. Clinics are selected to ensure representative sampling of the donor population. All donations with available plasma samples are tested by individual donation NAT at the American Red Cross laboratory in Gaithersburg, MD, using the **cobas**® HEV test for use on the **cobas**® 6800/8800 System. This test is not currently approved in Canada or the USA. All NAT-reactive donors are questioned concerning risk factors for recent HEV infection, undergo confirmatory testing (alternate NAT, viral load, genotyping and serologies), are notified by letter, and deferred from donating for 6 months; in-date products collected from the donor, and any frozen red blood cells or plasma from the previous 6 months are destroyed. Recipients will be traced in the event of any products transfused in the previous 6 months.

Results: As of the end of February 2017, 6 of 26,749 (12,256 CBS, 14,493 HQ) tested samples with valid results have been found HEV-NAT reactive; 5 donors have been confirmed by further testing to date. Of the 6 donors, five were from Quebec and one from Nova Scotia. Five were males and one female; ages ranged from 21 to 70 years. Only one donor reported non-specific symptoms (fatigue). In terms of risk factors: four ate pork (including two who ate pork liver), three ate shellfish, two ate venison, and two drank well water. One donor had no identifiable risk factor.

Summary/Conclusions: The prevalence rate of acute HEV infection in this donor population appears to be around ¼,460. The data from this study will contribute important information to the ongoing risk assessment concerning the risk of transfusion-transmitted HEV infection in Canada.

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HEPATITIS E VIRUS INFECTION: INCIDENCE AND EPIDEMIOLOGICAL CHARACTERISTICS OF POSITIVE BLOOD DONORS IN CATALONIA (SPAIN)

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Background: Hepatitis E virus is an emerging threat in blood safety. In a previous pilot study performed in 2013 in 10,000 blood donors from Catalonia, we observed an HEV RNA incidence of 1 positive blood donation per 3,333 using an individual NAT test.

Aims: To extend the HEV RNA incidence study in our blood donors population and determine viral and demographical characteristics of HEV positive blood donors.

Methods: HEV RNA was tested in samples consisting of 48 pooled blood donations (MP-48) using an in-house real-time RT-PCR. The analytical sensitivity of the method was determined by Probit analysis using HEV WHO International Standard (PEI code 6329/10) and was 7.9 IU/ml (CI 95%: 5.5–16.6 IU/ml) which corresponded to a sensitivity of 377 IU/ml (CI 95%: 265–799 IU/ml) in individual donation. Blood donations included in any RT-PCR initially reactive MP-48 were further tested individually with the automated PROCLEIX HEV ASSAY (Grifols, 7.89 IU/ml at the 95% detection probability). HEV IgG, HEV IgM (recomWell HEV IgG /HEV IgM, Mikrogen) and liver function tests were obtained in HEV RNA positive blood donations. Genotyping was performed by ORF2 sequencing.

Results: From April 2016 to February 2017, 80,334 blood donations were analysed in 1690 MP-48, which corresponded to 33% of all blood donations. Thirty-one MP-48 were RT-PCR initially reactive, and 14 were considered false-positive. Seventeen blood donations were confirmed positive for HEV RNA. Incidence of HEV RNA was thus 1 in 4725 blood donations during this period (CI 95%: 1/2941 to 1/8333). HEV RNA positive blood donors were 11 male (65%) and 6 female with a mean age of 49 ± 8 years. The viral load ranged between 40 and 450,000 IU/ml and in 33% of positive donations viral load was below the method sensitivity (377 IU/ml). Most of HEV RNA positive blood donors (76%) were negative for HEV IgG/IgM at blood donation, and only 3 of them presented mild transaminases alteration. Genotype was obtained in 6 of them and was in all cases type 3 genotype (5 were genotype 3f, 1 genotype 3i). Follow-up sample was obtained in 12 HEV positive blood donors (median 17 days post donation) and in all donors seroconversion to HEV IgG/IgM was observed. Transaminases became normal in the follow-up sample in the 3 donors mentioned above, were higher in 4 (one of them with acute hepatitis symptoms), while in 5 cases transaminases remained normal. Viral load was higher than at initial positive blood donation in 3 cases, had decreased in 6 cases but was still detectable, and was negative in 3 cases.

Summary/Conclusions: In Catalonia, HEV RNA incidence was 1 out of 4,725 blood donations during the study, in accordance with results obtained in 2013 (1 HEV RNA positive donation per 3,333) and thus confirming the virus circulation in healthy blood donors. Should the method be applied to all blood donations, we expect to intercept annually in our region more than 50 HEV RNA positive blood donations.

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HEPATITIS E VIRUS: INVESTIGATION IN NORTH ITALIAN BLOOD DONORS AND ESTIMATE OF TRANSFUSION RISK

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Background: Hepatitis E virus (HEV) is a major cause of acute hepatitis worldwide, and a possible threat to transfusion safety. Recent data from Europe showed a HEV IgG prevalences of 6.8% in German blood donors, 27% in Dutch blood donors, and 52% in an hyperendemic area in the South of France.

Aims: The aim of this study was to determine the prevalence of anti-HEV reactivity and HEV viremia in Italian blood donors, in order to estimate the risk of transfusion transmission.

Methods: Nearly 10,000 samples were collected from anonymized, unpaid donors at the "Lecco processing and validation blood center" (Lombardy, Italy) in 2016. Samples were tested individually (individual-donation nucleic acid test [ID-NAT]) for HEV RNA using the Procleix HEV assay (95% limit of detection 7.9 IU/ml). Initial TMA-reactive samples were retested and considered positive if the retest result was reactive. For the serology study, a subset of 2000 donations was tested for HEV IgG using DiaPro HEV ELISA kit (Diagnostic BioprobesSrl, Milano, Italy). HEV IgG and IgM were analyzed in ID-NAT positive samples at the time of donation and in the follow up, collected one year after the index donation.

Results: The prevalence of IgG anti-HEV in north Italian blood donors was 7.4%. Nine out of 9,726 donor samples gave reactive values by the ID-NAT assay for HEV RNA. Among them, only one sample was confirmed to be reactive in additional TMA tests. None of the 9 HEV RNA initially reactive samples had circulating IgM or IgG antibodies against HEV. In the follow up, only the repetitive reactive donor shewed a IgM and IgG seroconversion, indicating primary HEV infection. Therefore, we estimated that the risk of receiving a potentially infectious blood unit is of 1:10,000 (upper bound of the 95% confidence interval, 1:1,700).

Summary/Conclusions: Anti-HEV reactivity, indicating a previous infection, was found in 7.4% of subjects admitted to blood donation in our area. We also identified a viremic blood donation, indicating that the risk of transmitting the infection through blood transfusion, although small, is not negligible. The clinical impact of HEV infection among blood recipients remains to be assessed. These data need to be considered when deciding a national policy for preventing HEV transmission.

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LACK OF EVIDENCE FOR THE TRANSMISSION OF HEPATITIS E VIRUS (HEV) BY COAGULATION FACTOR CONCENTRATES BASED ON SEROPREVALENCE DATA

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Background: Hepatitis E virus (HEV) can be transmitted by blood components from a single donor by transfusion. Whether HEV infection can also be transmitted by coagulation factor concentrates remains unclear.

Aims: To compare HEV seroprevalence in blood donors and patients with coagulation disorders after administration of coagulation factor concentrates.
Methods: Archived samples from whole blood donors and patients who had received coagulation factor concentrates were investigated for the presence of anti-HEV IgG using two ELISAs. Western blotting was used to confirm the positive samples that showed reactivity in both ELISAs. The seroprevalence between donors and recipients was compared.
Results: Sixty-eight of 357 blood donors (19%) presented IgG antibodies against HEV. Two of 92 patients who had received coagulation factor concentrates (2.2%) and 1 of the 69 patients who had received plasma-derived products (1.5%) tested positive for anti-HEV IgG. The seroprevalence of HEV in the patient group was significantly lower (P = 0.038) than that in the donor group. The two positive patients were a 72-year-old man treated with plasma-derived products and a five-year-old girl treated with a recombinant coagulation factor concentrate.
Summary/Conclusions: HEV seroprevalence was significantly higher in the blood donors than in the patients with a history of coagulation factor concentrate administration. In one of two patients with detectable anti-HEV IgG antibodies, the coagulation factor concentrate was not the probable source of infection. Our data suggest that HEV is efficiently inactivated during the manufacturing process of coagulation factor concentrates. Thus, testing for the presence of HEV RNA in plasma donated for the preparation of coagulation factor concentrates may not be necessary.

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SEROLOGICAL EVIDENCE OF HEV INFECTION IN BLOOD DONORS IN LAGOS NIGERIA – A PILOT STUDY

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Background: Hepatitis E is a common infection in developing countries like Nigeria because of poor sanitation and weak public health facilities. In Nigeria, HEV IgG prevalence as high as 42.7% has been reported in the general population but few studies have been carried out to determine HEV prevalence in blood donors.
Aims: To characterize the seroprevalence of anti-HEV IgM and IgG in blood donors in Lagos State, Southwest, Nigeria.
Methods: Sera from 151 donors were screened for anti-HEV IgM and IgG by enzyme-linked immunosorbent assay (ELISA). Data were expressed as the mean ± standard deviation and all statistical analysis was performed using SPSS version 23.0 statistical software (SPSS, Inc., Chicago, IL, USA) where P-value of 0.05 was accepted as statistically significant.
Results: In this study 151 blood donors were enrolled, including 140 (92.7%) males and 8 (5.3%) females. Their age ranged from 19 to 55 years (mean 31.01 ± 7.49 years; median 30 years).
Overall seroprevalence for HEV of 6.6% was determined. HEV IgG had a significant prevalence of 5.3% as against HEV IgM 1.3% (P = 0.00). Higher prevalence was found among males compared to females. A higher HEV detection rate was observed in the younger age group with 80% (8/10) of the anti-HEV positive donors aged 20 – 40 years old (P = 0.9). In this study, no significant association was observed (P > 0.05) between seropositivity and the risk factors associated with HEV infection.
Summary/Conclusions: Although this study revealed a low seroprevalence of HEV infection in the population evaluated, the results showed evidence of past and active HEV infection in blood donors from Lagos State, Southwest Nigeria, and underscores the need of further studies to clearly define the potential risk to recipients of blood transfusion nationally.

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Abstract has been withdrawn.

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PROSPECTIVE STUDY OF PREVALENCE OF HEPATITIS E VIRUS IN DONORS FROM NORTH-SPAIN

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Background: Hepatitis E virus (HEV) in a healthy population usually causes a mild or subclinical infection. However, in immunosuppressed patients and pregnant women can cause fulminant hepatitis.
Although the main route of transmission is fecal-oral via contaminated water, cases transmitted by transfusion have been reported as well as for food consumption (raw pork meat, viscera or seafood, etc).
In Spain, these foods are frequently consumed so it may be a possible route of transmission. There is a risk that the donor is asymptomatic at the time of donation and thus can transmit the HEV to the patient through the transfusion and lead to fulminant hepatitis in immunocompromised recipients.
Aims: The few studies carried out in Spain, makes the current situation in our country unknown. From the Transfusion Centers of the North of Spain, we propose to carry out a prospective prevalence study with the objective of knowing and characterizing our donors and assess the impact of our blood components with the aim of improving transfusion safety.
Methods: A working group was formed: "Ateneo Group" composed of 6 Transfusion Centers of the North of Spain.
The donor signed an informed consent where risk eating habits were collected:
1. Consumption of products of porcine origin/hunt/homemade slaughter
2. Consumption of raw meat (sausage) or undercooked meat.

Sample size: 17,689 donors collected in the period between May and September 2016, have been distributed in the following way:
1. Transfusion Center (T.C.) Castilla y Leon: 7,088 (samples sent)
2. C.T. Aragon: 3,606
3. C.T. Asturias: 3,274
4. C.T. Cantabria: 1,337
5. C.T. Navarra: 1,336
6. C.T. La Rioja: 1,048

The analysis were centralized in 'Castilla y Leon', an additional tube of 10 ml of anticoagulated blood was extracted, the plasma was separated and frozen at −20°C in 24 h. The tests were carried out with less than one month from the extraction with NAT technique in the cobas 6800 equipment Roche.
Serology, in Abbot auto-analyser for positive cases.
Results: Of our study population, 63% were men and 37% were women.
The median age is 48 years for men and 46 for women.
As for the food history, 98% have risk habits and only 2% do not.
Of the 17,689 samples analyzed, only one was positive HEV-RNA and serology IgG positive. A month after, it was repeated and HEV RNA was negative.
The prevalence of HEV in donors is 1/17,689.
Hemovigilance was performed, the CHD was transfused, the patient was NAT negative, the plasma was discarded and the buffy coat was not used.
Summary/Conclusions:
1. There is a high consumption of risk foods (98%) in our donors.
2. The prevalence in our population is very low compared to the results of other studies, so we do not have enough information to make the decision of whether to carry out the test on our blood donors.
3. We will complete our study in a second phase with fresher samples and with serology.

Immunohaematology

Red cell immunology: Serology

P-410

EVALUATION OF ANTI-A AND ANTI-B TITERS IN IRANIAN BLOOD DONORS WITH O BLOOD TYPE AND COMPARISON OF IMMUNE RESPONSES IN HIGH TITER AND NON-HIGH TITER DONORS

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Background: ABO Blood group antibodies naturally exist in normal individuals. Some incidents of intravascular hemolysis have been reported after transfusion of blood products due to a minor ABO incompatibility. Group O blood donors are very important to transfusion medicine and constitute the majority of donors. Large volume of ABO antibodies in blood components increases the risk of acute hemolysis transfusion reactions in recipients.
Aims: To enhance the safety of blood products we focused on screening plasma for high titer ABO isoagglutinins and determining the frequency of high titers anti-A and B as well as a critical titer for these antibodies. In addition, antibodies are important part of the immune system and provide a valuable paradigm for the study of different aspects of immune responses.
Methods: around 400 Blood samples were obtained randomly from voluntary O blood donors in Iranian Blood Transfusion Organization. Anti-A and B titers were determined within the samples. The diluted sera were tested by using tube and gel card methods. A critical titer and the standard method were introduced. 11 Samples with titer ≥1,024 and 11 samples with titer ≤128 were selected and tested for total IgM, IgG, IgA, C3, C4, CH50, proliferation assay by using PHA and LPS, lymphocyte markers, total protein and albumin, ASO, antibody screening, CBC, anti-microbial antibodies, total IgE level, and cytokine production by real-time PCR.
Results: Majority of titers obtained from tube test were 128 in RT and 256 in AHG tube test. The critical titer was determined as 256 and the samples with titer more than that introduced as possibly dangerous titer. Prevalence of high titers among O blood donors was around 30% that had at least one of anti-A or anti-B with titer more than 256 in tube test and 7% had merely high titer IgG in gel cards. The frequency of high titer antibodies in RT method were 10.3% for anti-A, 5.6% for anti-B, and also in AHG method 23.5% for anti-A and 12.3% for anti-B in tube test. The high titer probability has not a significant correlation with age and gender. Evaluation of total IgG, total IgM, % gamma globulins, IL2 and IL4 secretion, IgG2 subclass, and lymphocyte proliferation showed statistically significant differences between high titer and non-high titer groups so that all values were higher in high titer group. The rest of the tests didn't disclose any significant differences.
Summary/Conclusions: This study confirms that determining a critical titer for ABO blood group antibodies in blood product can be considered to increase safety in transfusion medicine. Regarding the fact that the titers of anti-A and B are concerned with environmental factors as well as genetics, it is essential to determine the prevalence of high titer donors and the critical titer in each population. Besides, presence of high levels of isoagglutinis in a person is not limited to the immune responses against polysaccharide antigens and may result in further responses like mass antibody production, class switching, lymphocyte proliferation, or cytokine production in a high responder person.

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EVALUATION OF ANTIBODY SCREENING FOR TYPE AND SCREEN: COMPARISON BETWEEN MICROCOLUMN AND SOLID PHASE ASSAYS

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Background: In Type and Screen practice (T&S) the method for detecting irregular antibodies assumes a leading role to identify the presence of alloantibodies in patients blood at the time of pretransfusion testing.
Aims: In our study we compared two new screening assays with the method in use in our center, in order to select the best one for the implementation of the T&S process.
Methods: Pretransfusion antibody screening was performed in patients candidate to transfusion therapy due to elective surgery with 3-cell panel microtube column agglutination system (ID-DiaCell I, II, III, Diamed), the system currently in use at our laboratory and with one new 4-cell panel solid-phase system (Capture-R Ready Screen, Immucor) and one 4-cell panel microtube column agglutination system (Serascan Diana 4, Grifols). In positive samples the specificities of the antibodies were investigated using the appropriate panels: Capture-R Ready ID for Immucor, ID-DiaPanel for Diamed and Identisera Diana for Grifols. Samples with discrepant result between the different systems was investigated with all antibody identification systems.
Results: We analyzed 986 samples, 382 men and 604 females (age: 3–98 years, median: 63 years). We detected a negative result in 967 samples (98.1%) and a positive result in 8 samples (0.8%) with overall systems. In five patients we identified a single alloantibody: anti-Cw (2), anti-D, anti-Kell and anti-Jka. In one patient we identified the presence of cold agglutinins while in two patients it is not possible to identify an antibody due to pan-agglutination. Discrepant results were obtained in 11 samples (1.1%): 3 samples were positive with Diamed and Grifols, but negative with Immucor, 7 samples were positive with Immucor, but negative with Diamed and Grifols and one sample was positive with Immucor and Grifols, but negative with Diamed. In these samples the identification of antibody specificity gave positive results in only two patients. We found an anti-M antibody in a sample with a positive result at the microtube column agglutination systems. Then, we found an anti-Kpa antibody in a sample with a positive result at the 4-cell panel systems. In all other cases, the positive result of antibody screening has not been confirmed by antibody identification. The sensitivity of Immucor and Grifols system was 90.0% and 100%, respectively. The specificity of Immucor and Grifols system was 99.3% and 99.8%, respectively.
Summary/Conclusions: All assays are able to detect clinically significant antibodies in patients' blood but choosing the best available method needs to evaluate the following considerations. First of all, the implementation of systems with a four-cell panel has the advantage of extending the red blood cell antibody screening to low frequency antigens which may represent a potential risk of transfusion reactions. Then, the limitation of solid phase is the inability to identify IgM alloantibodies (i.e. anti-M) that are optimally reactive at 4°C and rarely cause delayed hemolytic transfusion reactions. Therefore, the comparison of microcolumn agglutination and solid phase tests show that the microcolumn agglutination system has highest sensitivity and specificity.
In conclusion, it is conceivable that the implementation of a four-cell panel with a microcolumn agglutination technology might represent an optimal system for alloantibodies detection.

P-412

A UK NATIONAL EXTERNAL QUALITY ASSESSMENT SERVICE (UK NEQAS) PILOT FOR THE DIRECT ANTIGLOBULIN TEST (DAT) – AN ASSESSMENT OF SENSITIVITY BY TECHNOLOGY

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Background: Between July 2015 and January 2017, UK NEQAS sent 12 red cell samples to participants in the DAT pilot scheme, with the intention of establishing a full EQA scheme. These comprised 4 negatives (uncoated), 1 C3d coated and 7 IgG coated sets of cells, with varying reaction grades. 4 of the IgG coated samples had reaction grades of 2+, and data relating to these 4 samples has been collated for this study.
Aims: To assess the sensitivity of DAT by technology for the 4 IgG coated samples with an intended reaction grade of 2+ vs anti-IgG.
Methods: Participants were asked to process the DAT samples, as they would a similar clinical sample, within 7 days of issue and provide reaction grades and technology used via SurveyMonkey. The data from 200 UK and Republic of Ireland laboratories was collated and analysed for reported reaction grades vs polyspecific anti-human globulin (AHG), anti-IgG and anti-C3d by each technology. Overall, 792 sets of results

with reaction grades were included. Mixed field reactions, transcription or transposition errors, and where technologies were not provided, were removed from the data. Technologies with less than 12 users were not included in the comparison.
Results: The overall median result vs polyspecific AHG and anti-IgG was 2+ for all 4 samples. When tested vs polyspecific AHG, 24.8% and 50.0% of reported reaction grades in Bio-Rad (n = 206) and Grifols (n = 26) respectively, were stronger than the median, whilst 9.2% and 3.8% respectively, were weaker. Conversely, 52.6% and 29.4% of reported reaction grades in Ortho (n = 58) and tube (n = 18) respectively were weaker than the median, whilst 8.8% and 17.6% were stronger vs polyspecific AHG. A similar picture was noted with anti-IgG, with 27.2% and 42.3% of reported reaction grades in Bio-Rad (n = 303) and Grifols (n = 26) respectively were stronger than the median, and 9.3% and 7.7% respectively were weaker. Conversely, 50.0% and 42.1% of reported reaction grades in Ortho (n = 124) and tube (n = 19) respectively, were weaker than the median, whilst 10.7% and 10.5% respectively were stronger. This data includes 8 false negative reactions in Ortho (by 3 participants), 1 in Bio-Rad, 1 in tube and none in Grifols.
Summary/Conclusions: When compared to the median reaction grade of 2+, the data showed that a majority of Bio-Rad and Grifols users reported the grade as same or stronger vs both polyspecific AHG and anti-IgG, and that a majority of Ortho and tube users reported the reaction grade as the same or weaker than the median. The majority of the false negative reactions occurred in Ortho, although 6 of these were reported by one participant. From this data, we conclude that Bio-Rad and Grifols technologies demonstrate the greatest level of sensitivity for detecting a 2+ DAT (coated with IgG), and Ortho and tube a lower level of sensitivity.

P-413

A SIMULTANEOUS STANDARDIZATION OF SPRCA TECHNIQUE FOR TITRATION OF ISOAGGLUTININS (ANTI-A, ANTI-B) IN GROUP O INDIVIDUALS AND HEAT INACTIVATION METHOD FOR IGG TITRATION: A STUDY FROM INDIA

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Background: In O blood group individuals anti-A and anti B antibodies belong to both IgM and IgG class. Though, the proportion of IgG is small but they have potential to cause intravascular hemolysis. That's why they are pronounced as hemolysins. There are reports of hemolysis of recipient's red cells after transfusion of O group plasma to non-O group patients. Now a –days, in a tertiary healthcare center, isoagglutinins titration in ABO incompatible solid organ transplantation is a routine investigation.
Aims: (i) To establish/standardize SPRCA against conventional test tube (CTT) technique for titration procedure and reporting; (ii) To standardize heat inactivation method for IgG antibody titration.
Methods: This study was performed at a tertiary healthcare center in north India between August 2015 and Jan 2017. Anti A and anti B titer was done in O blood group individuals using CTT method described in AABB. Both IgG and IgM titers were done. SPRCA titration was done using fully automatic NEO (Gamma Immucor, USA). IgM titer was done using hemagglutination method using 96 well microplates. IgG titer was done using 96 well capture select plates. Two different assays were used for low and high titration. Automated serial dilution was performed in red cell coated strip. The titer endpoint was the reciprocal of the highest dilution yielding 1+ reaction strength. The strength of reaction was measured from 1+ to 4+ following AABB methods. CTT was considered gold standard for standardization of SPRCA for titration. For IgG antibody titer, heat inactivation method was standardized against DTT with CTT technique.
Results: Titration study was done on 650 group O voluntary donors. Majority were males (97%). CTT was more time consuming and cumbersome method than SPRCA (120 vs 60 min). For anti A IgG, considering 1+ reaction as the titer endpoint, we found that 50% individuals had titer below 128, 33% individuals had titer equal to 128 and remaining 17% had titer above 128 by CTT method while in SPRCA it was above 128 in 50% individuals. A similar trend was observed in case of anti B IgG antibody in which 41% individuals had titer above 128 with SPRCA while with CTT just 25% had titer above 128. Considering 1+ reaction as the titer endpoint, we could observe a poor coherence between SPRCA and CTT for both anti A and anti B IgG antibodies (SPRCA = 10%).But, when we chose 2+ reaction as the titer end point reaction in SPRCA (against the 1+ reaction strength in CTT) the coherence improved significantly to 88% cumulatively for IgG, anti A+ anti B (88%) and for IgM titer (83%). 34/40(85%) samples demonstrated titer equal to DTT treated serum samples while 2 samples showed titer one dilution higher. One sample demonstrated one dilution lower titer.
Summary/Conclusions: SPRCA is a sensitive, objective, time saving and reliable method for isoagglutinins titer. For IgG titration, heat inactivation is as reliable method as DTT for IgG titration.

P-414

A NOVEL ADJUNCT AUTOMATED BLOOD BANK METHOD TO MANAGE INTERFERENCE FROM THE MONOCLONAL ANTI-CD38 DRUG DARATUMUMAB

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Background: Daratumumab is a promising new therapy for Multiple Myeloma, and is also being trialed in various regimes for other CD38-positive malignancies. As it is an anti-CD38 IgG1 Monoclonal Antibody that recognizes CD38 on myeloma cells and there are also varying levels of CD38 on red cells, it demonstrates panreactivity with antibody testing in the Blood Bank, thus causing interference and therefore a delay in the provision of suitable red cells for transfusion. Some of the suggested solutions in the literature of using DTT, trypsin, α-chymotrypsin, or a panel made from cord cells are not feasible in the Australian setting with imminent complex and expensive In Vitro Diagnostic regulations.
Aims: Under these circumstances, our laboratory worked to develop a protocol that includes an automated Prepapainised panel as an adjunct investigational method in detecting underlying antibodies that are not destroyed by papain. As it is a saline and non-IAT method, it is not appropriate to be used alone to assure compatible units for transfusion, but our testing shows that some significant underlying antibodies can be detected by this method.
We also refer samples for genotyping, and if the patient has already commenced Daratumumab treatment, another enzyme method to investigate for underlying antibodies is performed at our referral laboratory.
Methods: Initially, plasma samples from patients on Daratumumab were run on our automated instrument with an 11-cell panel by the IAT/CAT method. The samples were then run on the same instrument with a Prepapainised panel on saline cards. A DAT and titres were also performed.
The samples were then spiked with known antibodies and run in the same manner. A variety of known IgG antibodies including those from the RH, KEL, JK, FY, M and S systems and some combinations were tested.
Also, red cells from our Daratumumab patients who had been on the drug for over 12 months and were Group O with a known genotype were tested against other Daratumumab plasma samples and also Daratumumab patient plasma that was spiked with known antibodies.
Results: The initial samples showed panagglutination on our standard IAT/CAT platform, but were negative with the Prepapainised panel. However, the samples spiked with known antibodies which also showed panagglutination on the IAT cards, when run with the Prepapainised panel, were positive with the cells that contained the appropriate matching antigen, except for those antigens known to be destroyed by a papain enzyme method, e.g. FY and MNS.
All Daratumumab plasma samples spiked with known antibodies were positive with the Daratumumab patient red cells that expressed that antigen, and negative against those without the corresponding antigen.
Summary/Conclusions: We have shown that even though Daratumumab patients will show panagglutination with standard testing in the Blood Bank for detecting underlying antibodies, by using an easy, automated method of a Prepapainised panel, a good proportion of clinically important antibodies may still be detected. Although it is not a complete solution, it is a very helpful and economical adjunct method to assist us in dealing with an important emerging issue for the laboratory, and has the added advantages that it is easy to use and available at all hours in a standard Blood Bank.

P-415

HIGH EFFECTIVENESS OF ANTIBODY SCREENING USING COLUMN AGGLUTINATION TECHNIQUE WITH SCREENING POOL O CELLS

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Background: Column agglutination technique (CAT) is very high sensitivity technology for antibody screening especially in indirect antiglobulin phase (IAT). Using CAT, alloantibodies could easily be detected with less turn-around time. However, false positive rate in weakly positive sample is still elusive and needed to be explored.

Aims: To evaluate the effectiveness of antibody screening protocol by screening Pooled O red cells using CAT technology.
Methods: Donor samples of National Blood Center (NBC), Thai Red Cross Society were recruited in year 2014 and subsequently analyzed using CAT (Ortho AutoVue Innova System, Ortho-Clinical Diagnostics Pencoed, United Kingdom) with screening pooled red O cells. Only weakly positive samples were repeated with screening pooled O red cells or 2-cell screening cells provided by the Thai NBC. Antibody identification was performed in weakly positive samples using a panel of 11 group O red cells (Thai NBC) with conventional tube test (CTT) and ORTHO Antibody Identification Panels (ORTHO RESOLVE® Panel A) using CAT technology.
Results: A total of 10,103 Thai blood donor samples were included in this study. 9,883 samples (97.82%) showed negative antibody screening, 53 samples (0.53%) showed strongly positive antibody screening (grading 1–4) and 167 samples (1.65%) showed weakly positive antibody screening (grading 0.5). Among weakly positive samples, 121 samples were repeated with screening pooled O red cells and showed positive result (72.46%). While 146 samples were repeated with 2-cell screening cells and showed positive result (87.43%). The false positive rate (antibody screening positive but antibody identification negative) among screening pooled O red cells and 2-cell screening cells was 10.74% and 13.70%, respectively. The incidence of alloantibodies detected by Thai NBC Panel cells and ORTHO Antibody Identification Panels were 89.66% and 74.14%, respectively. The most commonly found antibodies in weakly positive sample were anti-Lewis: 58.18% by NBC Panel cell and 84% by ORTHO Antibody Identification Panels.
Summary/Conclusions: In this study, we demonstrated that when using the antibody screening protocol (CAT) and pooled O red cells (following AABB guideline), the weakly positive samples needed to be remeasured to exclude the possibility of a false positive result. This strategy can avoid unnecessary loss of precious blood products from donors (10%>13%). These findings might be helpful for managing weakly positive results by CAT in both hospital and blood donation center settings.

P-416

EVALUATION OF THE CROSSMATCHING TECHNIQUES IN KOREA: MANUAL TUBE TECHNIQUE WITH ANTIGLOBULIN PHASE VS COLUMN AGGLUTINATION TECHNIQUE

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Background: Crossmatching (CXT) is an essential part of pre-transfusion testing and the antiglobulin phase must be included in CXT to detect unexpected antibodies in intended recipients. Although the manual tube technique with antiglobulin phase (AHG Tube) is widely used in blood bank laboratories for CXT in Korea, the column agglutination technique (CAT) has become increasingly popular. Biannually the Transfusion Medicine Korean External Quality Assessment Scheme (KEQAS) provides three samples, two negative and one positive as survey materials for CXT. In Korea, a comprehensive evaluation of CXT techniques is still lacking.
Aims: The aim of this study was to evaluate the two CXT techniques, AHG Tube and CAT that are currently used in blood bank laboratories in Korea.
Methods: Positive crossmatch samples were prepared by the addition of polyclonal anti-D (DiaMed GmbH, Cressier, Switzerland) to samples of blood group O fresh frozen plasma. A total of 534 and 521 positive test results for AHG Tube and/or CAT were included in two separate trials. Hemagglutination reaction strengths in tube and CAT were graded using a 0 to 4 scale (0.5, 1, 2, 3, 4). The grading score was used to compare the average grades between the two techniques at the same laboratories and analyzed using a paired *t*-test. The percent of correct answers between AHG Tube and CAT were analyzed using the chi-square test. Data from the 2016 Transfusion Medicine KEQAS were analyzed.
Results: 72.5% of participants used the AHG Tube CXT in the first trial and 71.5% in the second. Similarly CAT was used by 19.3% of participants in the first trial and 20.2% in the second. Both techniques were used by 8.2% and 8.6% respectively in each trial. The percent of correct answers for AHG Tube and CAT was 87.7% and 97.3% ($P = 0.0012$) for the first trial and 94.5% and 97.3% ($P = 0.185$) for the second trial. The average (± standard deviation) grades for AHG Tube and CAT were 2.0 (±0.8) and 2.5 (±0.9) ($P < 0.001$) for the first trial and 2.4 (±0.9) and 3.0 (±0.7) ($P < 0.001$) for the second trial. 50.0% and 26.7% respectively showed the same grading in each trial, however 45.5% (4.5%) and 64.4% (8.9%) were more sensitive in CAT (AHG Tube) than in AHG Tube (CAT). Six laboratories were less sensitive using CAT compared to AHG Tube. Three laboratories failed to detect hemagglutination in CAT during both trials.
Summary/Conclusions: Although most blood bank laboratories in Korea are using AHG Tube technique, our data suggest that CAT was more accurate and sensitive than AHG Tube for CXT, especially in the sample from the first trial with a lower antibody titer. Some laboratories were suspected of incorrect use of CAT therefore further education is warranted for the improvement in the techniques used for CXT.

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METHOD VALIDATION OF ANTIBODY SCREENING BY INDIRECT ANTIGLOBULIN TEST ON FULLY AUTOMATIC MACHINES

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Background: Screening of donor plasma for unexpected red cell antibodies is considered the international standard. The Thai Red Cross National Blood Service currently utilizes many different methods for screening of unexpected red cell antibodies in donors, including automated methods – ORTHO AutoVue Innova, Bio-Rad IH-1000 and Diagast Qwalys 3 – and conventional tube test. An Indirect Antiglobulin Test (IAT) is performed, regardless of technology used.
Aims: To evaluate detection of unexpected red cell antibodies by IAT method with different automated IH analysers. The data produced by the study is intended to be considered for selection of an appropriate automated analyser for the National Blood Service and National Blood Centre.
Methods: This study evaluated the sensitivity and specificity of each automated IH analyser investigated – ORTHO AutoVue Innova, Bio-Rad IH-1000 and Diagast Qwalys 3 – as compared to Conventional Test Tube (CTT) IAT. Sensitivity testing was determined by testing known positive antibody plasmas in each test system. A total of 134 samples were tested, with specificities of anti-E (13), anti-D(4), anti-e(1), anti-Mia+anti-E(4), anti-Dia(1), anti-Mia(63), anti-Lea(17), anti-Leb(12), anti-Lea+anti-Leb(16), anti-Mia+anti-Lea+b(1), anti-Mia+Unidentify Ab(1), anti-P1(1). Specificity was determined by testing 1,070 random negative samples (by CTT) from the routine daily workload at the National Blood Service.
Results: Compared to CTT, the calculated sensitivity for each analyser was AutoVue Innova was 94.03% (126/134), IH-1000 was 64.93% (87/134) and Qwalys 3 was 70.15% (94/134). Calculated specificity for each analyser was AutoVue Innova 99.63% (1066/1070), IH-1000 99.91% (1068/1070) and Qwalys 3 99.63% (1066/1070).
Summary/Conclusions: The study found that AutoVue Innova, IH-1000 and QWALYS3 show sensitivity of 94.03%, 64.93%, 70.15% respectively, with a specificity of 99.63%, 99.91%, and 99.63% respectively compared to manual CTT (IAT). As different IH analysers have different sensitivity and specificity, laboratories should carefully evaluate the different.

P-418

Abstract has been withdrawn.

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AUTOMATED TITRATION OF ALLOANTIBODIES ON THE NEO® PLATFORMS

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Background: The determination of alloantibody titers can be important in a variety of cases. For example, titers of anti-D or other clinical significant IgG antibodies determine the risk of HDFN (hemolytic disease of the fetus and newborn) and to monitor changes in antibody strength over time can be important during pregnancy. It is also relevant to donor/patient characterization in incompatible blood transfusion and blood group incompatible solid organ transplantation. Currently, most

titrations are performed manually by tube technique or in Column Agglutination Technique (gel) which is error-prone and time-consuming.
Aims: The aim of the study was to demonstrate performance of IgG specific automated titrations on Immucor's Galileo NEO® and NEO® Iris platforms using Capture® technology with the potential of employing a range of different sources of cells.
Methods: Patient, pregnancy and donor samples with known alloantibodies were tested in the low (1/1 to 1/128) and high titer (1/16 to 1/4096) antibody titration assay on the Galileo NEO® and NEO® Iris. Titers of alloantibodies tested on donor cells as well as Immucor's Panoscreen® cells were compared to investigate whether the usage of different cells leads to different titers.
Results: The results demonstrate different IgG antibodies can be titrated on the Galileo NEO® and NEO® Iris, including anti-D, anti-K, anti-S and many more. Moreover, usage of different cells to determine good titration reproducibility, i.e. anti-D and anti-K no titer change of more than 1 doubling dilution and Anti-S no titer change of more than 2 doubling dilutions was identified. Interestingly, titers differ if homo- or heterozygous cells are used for anti-c or anti-E.
Summary/Conclusions: The prototype automated Galileo NEO® alloantibody titration assay is able to titer a wide range of antibodies and demonstrates good reproducibility across different cells.

P-420

COMPARISON OF THE TUBE TEST AND COLUMN AGGLUTINATION TECHNIQUES FOR ABO ANTIBODY TITRATION IN HEALTHY INDIVIDUALS

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Background: Measuring the ABO antibody (Ab) titer is important in ABO-incompatible transplantation. The tube test (TT) technique is the traditional method used to perform titration studies. Recently, a fully automatic antibody titration assay using the column agglutination technique (CAT) was developed. This assay is superior in objectivity and reproducibility. However, few previous study has compared the TT technique with fully automatic CAT for ABO antibody titration.
Aims: To compare fully automatic CAT with the TT technique for ABO antibody titration.
Methods: We studied 30 healthy individuals (10 with blood group A, 10 with group B, and 10 with group O). All subjects were informed of the purpose and methods of this study, and they provided written informed consent. ABO antibody titration was performed using the anti-human globulin TT technique and CAT in the presence or absence of dithiothreitol (DTT).
The highest dilution point showing 1+ agglutination according to the AABB technical manual for TT was considered as the titer.
Results: The consistency between the TT technique and CAT for ABO titration was better in the presence of DTT. If the end point of titration was defined as 1+ agglutination in CAT similarly to that in the TT technique, the concordance of the ABO titrations between the techniques in the presence of DTT was poor where the kappa value was 0.2567 ($P > 0.001$). On the other hand, if the end point of ABO titration was defined as weak (W)+ agglutination in CAT independent of the TT technique, the concordance in ABO titration between the techniques in the presence of DTT was good where the kappa value was 0.3863 ($P < 0.001$).
Summary/Conclusions: Thus, we suggest that the end point should be changed from 1+ agglutination to W+ agglutination when using CAT in the presence of DTT to achieve a better concordant outcome with TT. The superior concordance between the TT technique and CAT was achieved probably because DTT added to CAT diminished IgM antibody activity and resulted in better detection of coexisting IgG antibodies. We conclude that 1+ agglutination in the TT technique could be equivalent to W+ agglutination obtained in CAT. Thus, we believe that CAT will be more reliable for ABO titration in an automatic manner than the TT technique in the future laboratory tests.

P-421

Abstract has been withdrawn.

P-422

LEVEL OF EXPRESSION OF CD38 ON RED CELLS ASSESSED BY QUANTITATIVE FLOW CYTOMETRY

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Background: It is a common occurrence that when daratumumab is present in a patient sample, it interferes with the antibody screen test, although the patient him-/herself is rarely DAT positive. It is assumed that this interference is caused by daratumumab binding to CD38 on the test cells. However, little is currently known about the actual level of expression of CD38 on red cells.
Aims: (i) To use quantitative flow cytometry to quantify the expression of CD38 on red cells from healthy blood donors and red cells from a patient in treatment with daratumumab, and (ii) to quantify the reactivity of daratumumab with red cells from healthy donors.
Methods: Fresh samples of EDTA-blood drawn from blood donors and from a patient in treatment with daratumumab were stained with anti-CD38 or daratumumab. For staining with anti-CD38, samples were washed and adjusted to a 2.5% suspension. Ten microliters (uL) of mouse anti-CD38 (Becton Dickinson, clone HB7) was then added to 5 uL suspension. After incubation at 20C, samples were washed and 25 uL of 1:10 diluted goat-anti-mouse-F(ab)2-PE (RO480, DAKO) was added. After incubation at 4C, samples were washed and resuspended before being acquired on a flow cytometer (Becton Dickinson FACSCanto II).
For daratumumab staining, 100 uL 15% red cell suspension was added 100 uL of 1:25, 1:50 or 1:100 diluted pharmaceutic daratumumab (Darzalex, human IgG1, 20 mg/ml) and incubated at 37C. After washing and adjustment to 2.5% suspension, cells followed the protocol as for anti-CD38, except that they were stained with anti-human-IgG (MH-16, CLB), instead of anti-CD38.
To enable calibration of fluorescence signals in antibody binding capacity (ABC), a calibrated standard (DAKO QIFIKIT) stained with RO480 was run in parallel to all experiments. Data from sample mean fluorescence were used to calculate mean ABC-level of each sample. After conversion to ABC, background fluorescence, also in ABC was subtracted to yield net ABC values corresponding to specific staining with anti-CD38 or daratumumab.
Phosphate buffered saline was used for all antibody dilutions, washing and suspension of cells.
Results: Mean ABC of anti-CD38 stained red cells (n = 21) was 146 (10- and 90-percentiles, 98–209). For individual samples, CD38 appear to be evenly and narrowly distributed on the red cells. I.e., there do not appear to be a subset of cells with strong CD38 expression. Red cells from the one available patient in treatment with daratumumab, had virtually no CD38 expression (net ABC of anti-CD38 stained cells, 2). The ABC level of daratumumab stained cells (n = 1) were comparable to that seen with HB7, ABC levels of 205, 202 and 223 for daratumumab dilutions of 1:25, 1:50 and 1:100, respectively.
Summary/Conclusions: The level of CD38-expression on red cells of healthy donors as estimated by quantitative flow cytometry is at a level comparable with the + - ++ screen test reactions seen with samples from daratumumab patients. Patients in treatment with daratumumab appear to have lost red cell CD38-expression. This is in accordance with the observation, that patients in daratumumab treatment are rarely DAT positive, although they are screen test positive due to daratumumab.

P-423

Abstract has been withdrawn.

P-424

MANAGEMENT OF RED BLOOD CELL TRANSFUSION IN MULTIPLE MYELOMA PATIENTS TREATED WITH DARATUMUMAB

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Background: Daratumumab (DARA), an anti-CD38 monoclonal antibody, has demonstrated rapid, deep and durable responses in Multiple Myeloma (MM) even in relapsing and refractory patients. DARA interfere with blood compatibility testing, mainly with the procedures utilized to avoid allogenic-immunization.
Aims: To develop/review practical management of blood transfusion in patients under DARA; to evaluate the relevance of development of allogeneic-antibody against red blood cells (RBCs) in the MM patients.
Methods: DARA from two MM patients samples (DARAA and DARAB) was added to five plasmas with identified antibodies anti-D, -E, -K, -Jka, -Fya (respective titers 256;128; 128;64;64). The resulting aliquots were: A1(DARAA+anti-D), A2(DARAA+anti-Fya), A3(DARAA+anti-K), B1(DARAB+anti-E), B2(DARAB+anti-Jka), B3 (DARAB+anti-K). The following tests were performed: 1. R2R2, Fy(a+b-), K+ and Jk (a+b-) RBCs (IMMUCOR□), treated and untreated with dithiothreitol (DTT), were used for indirect-antiglobulin test (IAT) through the column-agglutination method. 2. K+ and K- cord-RBCs (no DARA interference because of CD38 absence) were utilized to confirm the presence of anti-K.3. K+ and E+ RBCs were utilized to evaluate the efficacy/viability of DTT reagent and the K antigen denaturation and E antigen preservation was confirmed. 4. LISS/Coombs Cards (Grifols□) were utilized to direct and indirect antiglobulin test and MDmulticard (Grifols□) to determine the basic extended phenotype (Fya/Fyb;Jka/Jkb;S/s).Finally, the presence of anti-erythrocyte irregular-antibodies was retrospectively assessed in MM patients transfused between January 2010 and December 2016.
Results: 1. In DTT untreated cells, IAT presented (2+) positivity which is consistent with DARA interference; In DTT treated cells, IAT positivity corresponded to the antibody effectively present on the aliquot: anti-D in A1, anti-Fya in A2, anti-E in B1 and anti-Jka in B2 [lower titers (64;8;32;32 respectively) than the originally determined on plasma samples – sensitivity compromised]. The detection of anti-K in A3 and B3 was not possible, consistently with the effect of DARA in K antigen denaturation. 2. Anti-K were detected using cord-blood RBCs in A3 and B3.3. DTT viability was confirmed by stating the denaturation of K and preservation of E antigens. 4. Basic extended phenotype determination was consistent with the genotype. Of the 211 MM patients reviewed, two (0.95%) developed allo-antibodies anti-erythrocyte – one of which was a poly-transfused terminal patient (at that time, not under treatment for MM) and the other with one isolated positive IAT (all following results were negative).
Summary/Conclusions: DARA targets CD38, which is relevantly expressed on erythrocyte surface (ES). DTT removes DARA from ES. Here, we confirmed that DTT treated RBCs effectively detect irregular-antibodies, even in the presence of DARA but the titre of these antibodies is on average lower in two dilutions. DTT also denatures K antigen, thus hampering the detection of anti-K with normal RBCs. To overcome this problem, we used cord-RBCs which allowed the detection of anti-K. Our MM patients retrospectively studied rarely developed allogenic-antibodies against erythrocytes. The clinical/laboratorial setting in which those allogeneic-antibodies were developed raise questions about their relevance. Therefore, given the low humoral immunity reported in patients with MM and IAT compromised sensitivity associated with the procedure, we question whether this laborious and time-consuming procedure to evaluate allo-immunization is necessary.

P-425

SEROLOGIC CHARACTERISTICS OF ANTI-PIPERACILLIN IN 12 PATIENTS WITH DRUG-INDUCED IMMUNE HEMOLYTIC ANEMIA

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Background: Piperacillin is a broad spectrum semi-synthetic penicillin. It is commonly used in combination with tazobactam, a potent inhibitor of many β- lactamases which causes non-immunologic protein adsorption (NIPA) onto red blood cells (RBCs). The combined antibiotic is active against many Gram-positive and Gram-negative bacteria. Several cases of immune hemolytic anemia (IHA) induced by piperacillin/tazobactam have been reported. Since 2012, it has been the most common cause of drug-induced immune hemolytic anemia (DIIHA) investigated in our laboratory.
Aims: We retrospectively reviewed data on patients suspected of having DIIHA caused by piperacillin/tazobactam whose samples were sent to our laboratory from November 2012 to March 2016 and in whom anti-piperacillin/tazobactam were detected. This report gives serologic details of the 12 cases of DIIHA due to piperacillin/tazobactam we have encountered during this period.
Methods: Patients' sera and acid eluates from patients' RBCs were studied for the presence of piperacillin/tazobactam antibodies. Direct antiglobulin tests (DATs) and tests with untreated and enzyme-treated RBCs that had not been previously treated with the drug (i.e., by the so-called "immune complex" method) were performed. Parallel tests with phosphate-buffered saline (PBS) added to patient's serum or eluate instead of drug, or with fresh normal sera instead of patient's serum or eluate, served as controls. To determine the immunoglobulin class of the reactive antibody, testing was repeated after incubation of the patient's serum with dithiothreitol (DTT) or PBS. Drugs tested included piperacillin and piperacillin/tazobactam.
Results: Nine (75%) of the 12 patients had antibodies to piperacillin or piperacillin/tazobactam detected by the tube and gel method. Two of them appeared to have anti-tazobactam which had to be confirmed by testing tazobactam separately. Drug antibody reactivity was enhanced by testing enzyme (trypsin)-treated RBCs. Eight (67%) of the 12 samples tested demonstrated positive DAT: 4 were reactive with both anti-IgG and anti-C3d, 2 with only anti-IgG and 2 with only anti-C3d. All of these were associated with anti-piperacillin/tazobactam showing a relative RH5 (e) specificity. Four eluates were obtained from DAT positive patients' RBCs: 2 contained anti-piperacillin/tazobactam and 2 were non reactive. Four patients presented with a negative DAT: anti-piperacillin was detected in one patient; NIPA due to tazobactam was suspected in three cases with pooled normal sera reactive against enzyme-treated RBCs in the presence of high concentrations of piperacillin/tazobactam.
Summary/Conclusions: Piperacillin/tazobactam dependent antibodies can cause severe intravascular hemolysis. Complement can usually be detected on the patient's RBCs. IgG, IgM or IgG + IgM activating-complement anti-piperacillin can be involved. The drug-dependent antibody and/or the drug-induced NIPA could have contributed to the patients' hemolytic anemia. A negative or very weakly positive DAT should not exclude investigation of piperacillin/tazobactam induced immune hemolytic anemia especially if the sample tested was not obtained during active hemolysis. Although DIIHA is not common, drug antibodies should be considered when other causes of the hemolysis have been excluded.

P-426

INVESTIGATION OF THE RELEVANCE OF A POSITIVE DAT WITH RESPECT TO THE DETECTION OF A DELAYED SEROLOGICAL TRANSFUSION REACTION DUE TO RED BLOOD CELL ALLOANTIBODIES

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Background: The direct antiglobulin test (DAT) is used in pre-transfusion samples to trace immunoglobulin (IgG) or complement on the surface of red blood cells (RBC). In recently transfused patients a positive DAT may be a first sign of a delayed serological transfusion reaction. DAT work up using elution procedures and highly sensitive antibody detection methods as indirect antiglobulin tests with enzyme treated test cells may identify novel alloantibodies not detected in routine antibody screening tests.
Aims: In order to investigate the diagnostic efficiency of the work up of a positive DAT we analyzed in a retrospective study 159,719 blood samples in 54,100 different patients (age above 28 days) covering a 4-year period.
Methods: In all samples a polyspecific DAT was performed using a gel card assay (Bio-Rad, Switzerland). If positive monospecific DAT (anti-IgG, anti-C3d) were obtained. In patients with newly identified positive DAT with anti-IgG or a significant increase of the agglutination strength with anti-IgG and a recent RBC transfusion within the prior 28 days a DAT work up including elution (BAG, Germany) and highly sensitive antibody detection methods as IAT using enzyme treated test cells was performed.

Results: 29,942 (19%) samples showed a positive polyspecific DAT. 85% were positive only with anti-IgG, 7.3% with both anti-IgG and anti-C3d and 6.5% with anti-C3d, respectively. Following our internal algorithm 2,866 (9.6%) samples were further investigated. In 40 samples DAT work up revealed 44 novel RBC alloantibodies not identified in routine antibody screening tests. The rate of informative positive DAT work up thus was 1.4%. Most frequent novel alloantibodies were anti-E (14) and anti-Jk(a) (11). In 39 (98%) of these agglutination strength with anti-IgG was equal or below 2+. In 26 of 40 the interval of a recent putative incompatible transfusion (with respect to the newly identified antibody) could be recorded. In 21 (81%) the interval was between 1 and 14 days, in 3 (11.5%) between 15 and 21 days and in 2 (7.7%) more than 21 days, respectively.
Summary/Conclusions: Positive DAT work up may reveal novel RBC alloantibodies reflecting a delayed serological or haemolytic transfusion reaction. In order to increase the predictive value of DAT work up we adjusted our algorithm. We suggest to perform elution studies in patients transfused within the past 21 days with a newly identified positive DAT with anti-IgG with an agglutination strength equal or below 2+.

P-427

Abstract has been withdrawn.

P-428

CASE REPORT: A PATIENT WITH ANTI WR(B) ANTIBODIES, AND A STUDY FOR THE FREQUENCY OF WR(A) ANTIGEN IN THE DUTCH DONOR POPULATION

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Background: The pre-operative laboratory testing before a knee operation showed a positive red blood cell antibody screen in a 70-year old woman. The serum was strongly reactive with all 3 screening cells. The plasma was reactive with all cells tested as part of the antibody identification protocol, without binding to the patient's own red blood cells. The direct antiglobulin test was negative.
Aims: Treatment of a test cell with Ficin, DTT (0.2 M), Trypsin or Chymotrypsin did not influence the reactivity of the patient's serum. This points to antibody against an antigen with a high frequency in one of the following blood group systems; Rh, Jk, Co, Xk, Di, H, LE, P, 0K, At, Jr, Emm, Lan and Vel. The plasma was reactive with all available test cells lacking an antigen belonging to one of these systems. Serologic typing of the red cells of the patient revealed the absence of the Wr^b antigen, an antigen with a very high frequency in the DI blood group system. Genotyping showed a homozygous mutation in the *DI* gene (1972G>A), predicting a Wr(a+b-) phenotype. The serum of the patient was not reactive with two Wr^b negative test cells. The presence of other antibodies was excluded by performing an allo absorption.
Methods: Because anti Wr^b antibodies are seldom found, there is not much information on their clinical relevance. In one case the biological activity was tested with a chemiluminescence assay, which showed a shortened lifespan of Wr^b positive red cells. Our patient did not need a transfusion, and is advised to join the Dutch rare donor program. Also family members are advised to become tested for the absence of Wr^b. The antithetical antigen of Wr^b is the low frequent antigen Wr^a. The frequency of the Wr^a antigen is variable in different populations: 0.04% to 0.12% (Daniels, Human Blood Groups, 2013). In 1955 van Lochem showed a higher frequency of Wr^a in the Dutch population (0.3%, Vox Sanguinis).
Results: The chance to identify a Wr^b negative donors is investigated with 154,000 Wr^a typing results of Dutch donors (source: E-progesa, Sanquin), in relation to the postal code area. The overall frequency of Wr^a in the Dutch population was 0.5%, hence, higher than in other populations. This leads to a frequency of 0.002% for Wr^b negative donors. The frequency of Wr^a was variable over the country: ranging from 0.11% to 1.5%. Two clusters with a higher frequency of Wr^a were detected: one in the south-western part and the other in the northern part of the Netherlands. The patient with the anti- Wr^b came from an area with a higher frequency of Wr^a (0.94%).
Summary/Conclusions: Given the Wr^a-antigen frequency, it may be possible to identify among Wr^a-positive donors, one or two Wr^b negative ones by serological screening with the serum of the patient or by genotyping.

P-429

IMMUNE COMPLEX FORMATION INVOLVING ETORICOXIB IN A PATIENT WITH ACUTE SEVERE ANEMIA

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Background: Drug-induced immune hemolytic anemia is rare, and a specialized laboratory is often required to provide optimal serological tests to confirm diagnosis. Etoricoxib, a selective inhibitor of cyclooxygenase 2 (COX-2) is increasingly used in pain relief, there have been a few cases reported of etoricoxib-induced immune hemolytic anemia. Immune complexes formed between some drugs and their respective antibodies attach weakly in a nonspecific way to Red Blood Cells (RBC). The bound immune complex activates complement, which may lead to hemolysis in vivo.
Aims: Demonstration of immune complex formation involving etoricoxib in vitro.
Methods: A 46 year old woman developed acute severe anemia one day after a single dose of etoricoxib 90 mg per os with a positive direct anti-globulin test and a weak positive indirect anti-globulin test. The identification of the anti-RBC antibodies was not possible due to panreactivity. There was no evidence of active bleeding, auto immune disease, infectious disease or paraneoplastic syndrome. The patient started steroid treatment and transfusion support with slow evolution of hemoglobin levels. For diagnosis, we used the American Association of Blood Banks Technical manual protocol using patient serum collected in three different moments: firstly we drew blood at patient admission in emergency department (less than 24 h after the patient ingested the drug); the second sample was collected one month after starting steroid therapy and the third sample one month after stopping the treatment with steroids (5 month after the first assay).
Results: We found strong positive reactions when the patient serum was tested with the drug either in the first measurement and the last. The second measurement also showed a positive reaction yet weaker. We also found a drug independent reaction in the first measurement. In all three assays the reaction was stronger when mixed with normal serum as complement source. All the reaction where stronger when examined for agglutination than when tested with polyspecific antiglobulin reagent.
Summary/Conclusions: The authors believe that the strong agglutination that occurred in the mixture of the drug and the patient serum indicates a drug/antidrug reaction. We believe that the drug independent reaction found in the first measurement was due to circulating drug in the patient serum. The fact that all the assays showed a stronger agglutination in the presence of normal serum may indicate a complement mediated reaction. The positiveness of the third reaction led us to believe the patient serum had immunological memory, however, this hypothesis should have been explored with more exhaustive serological testing.

P-430

TRANSFUSION IN A RARE CASE OF PARA-BOMBAY PHENOTYPE

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Background: Individuals with Bombay phenotype are characterized by the absence of ABH blood group antigens both on the surface of red blood cells (RBCs) and in secretions resulting from silenced mutations in *FUT1* (*h/h*) and *FUT2* (*se/se*) genes, respectively. In contrast, para-Bombay phenotype retains some H antigen on RBCs either induced from a weakly active (*H+weak/H+weak)* or completely silenced *FUT1* gene (*h/h).* The latter is mandatory linked with an active *FUT2* gene (*Se/Se* or *Se/se*) enabling synthesis of ABH-antigens in secretions which may be adsorbed from the plasma onto RBCs surface. The anti-H in para-Bombay individuals is usually weak and often does not react above room temperature.
Aims: A 61 year old Thai female with metastatic pancreatic cancer was hospitalized due to clinical deterioration with radiologically confirmed progression under palliative chemotherapy. A blood sample was referred to our laboratory for AB0 grouping.

Here we describe the serological and genetic work-up which revealed AB para-Bombay phenotype and subsequent patient transfusion management.
Methods: Standard serologic techniques were used to detect ABH on RBCs (Bio-Rad, Cressier, Switzerland; Biotest AG, Rupperswil, Switzerland). In addition, a very potent anti-A/B serum (Medion Grifols Diagnostics, Duedingen, Switzerland) was used to reveal traces of A and B antigens. Compatibility testing was performed using the indirect antiglobulin test (IAGT) at 37°C. Molecular ABO type was defined using a commercially available test kit (inno-train GmbH, Kronberg i.T., Germany). Sequencing was performed for coding exons of *FUT1* and *FUT2* genes.
Results: The routine anti-A, -B and -A/B failed to detect the respective antigens and, most notably, no H antigen was traceable. The RBCs showed only weak agglutination with the potent anti-A/B serum (Grifols). Only Anti-H, but no anti-A or anti-B, was identified in the serum. Initial ABO genotyping by sequence-specific priming (PCR-SSP) resulted in AB genotype. In order to confirm serological H-deficient phenotype a more detailed analysis was performed including sequencing of *FUT1* and *FUT2* which revealed an active secretor status but homozygosity for the *FUT1*01W.09* allele (c.658C>T,p.Arg220Cys). Latter is common in Taiwanese population and allows only weak expression of ABH-antigen on RBCs (Yu, Vox Sang., 1997), consistent with our observations.
Summary/Conclusions: In summary, our serological tests were in line with the characteristics of para-Bombay phenotype and confirmed by identification of the homozygous weakening mutation c.658C>T in the *FUT1* gene. However, if low level of ABH-antigens on erythrocytes is determined by partially active *FUT1* or normal secretor status is a matter of debate. Shortly after final diagnostics our patient developed acute gastrointestinal bleeding, requiring transfusion (Hb 53 g/l), fluid resuscitation and anticoagulation cessation. As we have no access to Bombay or para-Bombay blood in an emergency situation one A_1B whole blood unit with negative cross-match was transfused uneventfully and short-term stabilization was achieved. Due to the malignant primary disease her general condition further deteriorated and she died shortly thereafter under end-of-life care. In conclusion, we support the option to transfuse para-Bombay individuals with normal ABO blood group units, compatible by IAGT, when Bombay or para-Bombay blood is not available.

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COMPLETE DISAPPEARANCE OF PRE-EXISTING ABO ANTIGEN IN ACUTE ERYTHROID LEUKEMIA – A CASE REPORT

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Background: ABO antigens expression is expected to be constant throughout the life except in few clinical conditions. Hematological malignancies, especially the ones effecting the myeloid series can affect the expression of ABO antigens.
Aims: To present a case of missing A antigen in acute erythroid leukemia (AEL).
Methods: Miss. K, a 15-year-old girl came to our tertiary care hospital in Sept 2016 with complaints of fever, generalized weakness, and jaundice, was diagnosed with acute erythroid leukemia (M6a) with history of aplastic anemia and patient was started on induction therapy of Daunorubicin & Cytarabine. As she developed anemia during induction phase, a request for issue of one unit of Packed Red Blood Cells (PRBC) was made to the department.
Results: On routine workup her blood group was found to be O Rh D Positive on forward grouping and group A on reverse grouping. The red cells of the patient were agglutinated by anti-H reagents (extract Ulex europaeus) to the same degree as that of the control performed by the CTT. From the previous medical records, patient's blood group was A1 Rh D Positive. Absorption and elution tests with polyclonal anti-A revealed weak expression of A antigen on the RBC surface of the patient. She received three units of type O PRBCs (Packed Red Blood Cells) and several units' of single donor platelets of type A1. All the transfusion episodes were uneventful. Patient developed veno occlusive disease and/or sepsis on day 8 of induction along with neutropenic fever and succumbed to it.
Summary/Conclusions: The case signifies the importance of clinical details including historical blood group and a complete ABO blood grouping both forward and reverse. It is always helpful in reaching a conclusion and also guiding toward the appropriate transfusion support to the patient. This is the first documented case disappearance of A antigen on RBCs of a patient with acute erythroid leukemia. The change in RBC antigen, though occur rarely, are mostly encountered with hematologic malignancies especially of myeloid series. It is important for blood bank professionals and clinicians to be aware of the existence of this phenomenon.

P-432

WHEN BLOOD GROUPS ARE TRULY INTERNATIONAL: A CASE OF ANTI-JK3

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Background: The Kidd blood group system, discovered in 1951, consists of two antithetical antigens (Jka and Jkb) that define three phenotypes: Jk(a+b+), Jk(a+b-) and Jk(a-b+). The null phenotype, Jk(a-b-), is very rare and these individuals lack both the Jka and Jkb antigens.
Red cell antibodies against the Kidd system are formed secondary to sensitisation to missing antigens. They are clinically significant, since they can cause haemolytic transfusion reactions (HTRs) and haemolytic disease of the foetus and newborn (HDFN). Individuals with the null phenotype can form anti-JK3.
Aims: Awareness of rare antibodies that have been implicated in HTRs and HDFN.
Methods: We report a case of a 42 year old lady originally from the Philippines, G_2P_{1+0}, with no medical co-morbidities. The antenatal testing in her first pregnancy showed O RhD positive (R_1R_1), antibody screen negative. The antibody screening for the second (current) pregnancy was positive; with negative direct Coomb's test and auto-control. The antibody reacted equally with Jk(a+b−), Jk(a−b+), and Jk(a+b+) panel cells, and was identified as anti-JK3. This was further supported by the patient's phenotype: Jk(a-b-). The patient's partner was O RhD positive, Jk(a-b+).
The expected delivery date was end October 2016. However, the patient developed pre-eclampsia and spontaneous rupture of membranes towards the beginning of October; so an emergency C-section was performed. Transfusion cover was organised with two frozen red cell units from NHSBT [O R_1R_1, Jk(a-b-)]. Delivery was uncomplicated and transfusion was not required.
Results: The baby's haemoglobin and bilirubin were 206 g/L was 116 μmol/l respectively three days post-delivery; he was given phototherapy. The baby was typed as O R_1R_1, Jk(a-b+) and had a positive DAT with both IgG and C3d.
The anti-JK3 detected was most likely a result of sensitisation during the patient's first pregnancy (she was never transfused). The prevalence of Jk null phenotype is <0.01% among Caucasians and Blacks. However, it has a frequency of up to 0.9% in people of Polynesian and Finnish descent.
Patients with anti-JK3 require Jk(a−b−) red cells for transfusion. This can prove problematic to obtain due to the high incidence of Kidd antigens. Kidd antibodies are notorious for causing delayed haemolytic transfusion reactions. Their titres decrease over time, making their detection difficult during pre-transfusion testing. Upon being re-exposed to a foreign Kidd antigen, there is an anamnestic secondary response. Hence it is very important keeping good records of these antibodies at the Hospital Blood Bank and issuing the patient with an antibody card.
Summary/Conclusions: There are very few reported cases of anti-JK3 in pregnancy. Most result in mild HDFN, requiring phototherapy. Anti-JK3 should be considered when there is pan-reactivity with a negative auto-control, in a patient who had previous pregnancies or transfusion, particularly in certain ethnicities. Transfusion support may be difficult since suitable units are not easily sourced. Communication between blood banks, blood collection services and the patient's caring physicians are crucial for the effective management of the patient. More importantly, delivery of these patients should be planned in a hospital with cell salvage facility.

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CLINICAL CASE OF RH-POSITIVE RECIPIENT ALLOIMMUNIZATION (ERYTHROCYTES CATEGORY DVII) WITH DONOR'S RH-POSITIVE ERYTHROCYTES

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Background: Alloimmunization of recipients with donors' erythrocytes and immunological safety of transfusions of media containing erythrocytes largely depend on the correct testing of erythrocytes antigens. Routine testing methods of antigens of Rh rhesus system may not always predict alloimmunization during hemotransfusions. Genotyping of blood groups may reduce alloimmunization risks.
Aims: Retrospective immunohematologic study of a clinical case of Rh-positive recipient alloimmunization with donor's Rh-positive erythrocytes.
Methods: Patient K. (55 years old) with a diagnosis of inherited spherocytic anemia. Haemo-globin – less than 50 g/l, transfusion therapy is indicated for treatment. Phenotyping of erythrocytes and detection of antierythrocytic antibodies of patient K. were performed with the help of monoclonal reagents on a plane (Gematolog), in gel cards (Bio-Rad) and on the instrument "Galileo Neo" (Immucor). The specificity of antierythrocytic antibodies was defined on 11 cell panel (Bio-Rad). The category of rhesus D erythrocytes was established with the help of ID-Partial D Typing Set (Bio-Rad). Genotyping of partial rhesus D was performed with the help of SSP-PCR BAGene Partial D-TYPE (BAG).
Results: The erythrocytes phenotype of patient K. by all three methods was defined as A(II)CCDEekk. Antierythrocytic autoantibodies and alloantibodies have not been found. Transfusion of identical donor's red blood cell suspension of the phenotype A (II)CCDEekk was performed. 14 days later, antierythrocytic antibodies with specificity anti-D in the titer 1:16 were found. Retrospective phenotyping of erythrocytes and genotyping of patient K. with partial rhesus D enabled to establish the category of erythrocytes DVII rhesus.
During the single hemotransfusion of Rh-positive erythrocytes of the donor, alloimmunization of Rh-positive (DVII) of patient K. occurred with a generation of alloantibodies with specificity anti-D.
Summary/Conclusions: Recipients with partial DVII may form antibodies against the missing epitope of antigen D during single transfusions of the donor's D-positive erythrocytes. Routine phenotyping of erythrocytes does not allow identifying this category of erythrocytes as partial rhesus D and cannot predict alloimmunization by the rhesus system.

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IMMUNOHEMATOLOGY REFERENCE LAB IN SOUTH INDIA: TWO YEARS OF EXPERIENCE IN SOLVING CASES

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Background: Alloimmunization to red cell antigens resulting from pregnancy, transfusion, transplantation and sharing of needles causes problem in routine immunohematological testing.
At Regional Testing Center (RTC), Immunohematology reference Lab, Rotary TTK Blood Bank Bangalore we receive many cases with incompatible cross match, blood group discrepancy and post transfusion reaction from different blood banks & hospitals of Karnataka & nearby states. We also conduct training program to educate blood bank as well as health care staff about immunohematology problems & their clinical significance.
Aims: To analyze & evaluate the immunohematology reference cases received in last two years.
Methods: The RTC receives discrepant samples from various hospitals/blood banks of South India for Antibody screening and Identification, ABO typing discrepancies, Investigation of Positive DAT etc. The tests were performed by both tube technique as well as Solid Phase & Column Agglutination Technique according to the requirement. The advanced techniques like adsorption- elution & auto/ allo-adsorption were also performed as per the requirement.
Results: Out of 214 cases recorded from September 2014 to July 2016, 43 cases were requested for Resolution of ABO Typing Discrepancy, 18 cases for Investigation of Positive DAT, while remaining others were all requested for Antibody screening and Identification.
Of the total requested samples 63.5% were of female and 33.1% male patients/donors. Also, 43.4% of sample requested were from patient/donor of age group 20–40 yrs, 36.9% from >40 yrs old and 13% from pt./donor of age group <20 yrs.
Among ABO discrepancy cases 7 (16.2%) were found to be A2 subgroup and 3 (6.9%) were cases of weak D and weak A variants each. Similarly, only 2 (4.6%) of group discrepant cases were found to be of weak B variants. In remaining cases ABO discrepancy was solved by cold temperature enhancement technique.
Out of 154 samples for ABID, alloantibody/ies were found in 147 cases. It was found that Anti-D was most frequently detected antibody with 47 (31.9%) cases reported. Anti-M and Anti-E with 18 (12.2%) cases each reported made it to the second place. Anti-c, was found in 17(11.5%) cases, followed by Anti-C and Anti-Le^b with 11 (7.4%) cases each reported. Similarly, other antibodies identified were Lea 7(4.7%), K 5(3.4%); P1 & Fy^a 3(2.04%) each; s& Jk^a 2(1.36%) each; N, S and Fy^b 1(0.6%) each.
Similarly out of 38 DAT positive cases, 26 autoantibodies were confirmed which consisted of 6(23%) cold autoantibody, 5 (19.2%) warm autoantibody, 4(15.3%) mixed-type autoantibody and 12(46.1%) autoantibody of unknown specificity.
Summary/Conclusions: The analysis concludes that discrepant samples are mostly received for antibody identification which included significant number of DAT positive cases too. Frequency of alloimmunization was found highest in females with child-bearing age and the commonest antibody was anti-D. It is also observed that Anti-E is usually found in conjugation with other antibodies as 5 (55.5%) Anti-E was seen to be present in total 9 cases of multiple antibodies followed by Anti-c with 3 reported cases (25%).

P-435

MANAGING PATIENTS WITH ANTIBODIES TO HIGH-FREQUENCY ANTIGENS – OUR EXPERIENCE

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Background: An investigation of the antibody to a high-frequency antigen (HFA) is both very demanding and time consuming. Although some of the antibodies that are serologically difficult to identify are those with the least clinical significance (i.e. anti-Cs^a, -Ch, -Rg, -Yt^a, -JMH), many have been responsible for immediate HTR (i.g. anti-Vel, In^b), delayed HTR (i.g. anti-Kp^b, -Js^b), severe HDFN (i.g. anti-k, anti-RH17) or mild HDFN (i.g. anti-K18) where antigen negative blood should be transfused. While HFA-negative subjects are excessively rare amongst white people, transfusion management in patients with HFA alloantibodies is often difficult.
Aims: Our aim is to present patients with HFA alloantibodies with special focus on difficult diagnostics and transfusion support.
Methods: We investigated patients with alloantibodies directed against HFAs who were admitted or referred to our clinical hospital from January 2001 to January 2017. An antibody was assumed to be clinically significant if the specificity involved had been implicated in HTR or HDFN.
Results: A total of 30 patients had antibodies to HFAs. This corresponds to an incidence of 0.72 patients per 100,000 patients listed in our IT system per year. Among these patients there were 9 pregnant women. The most frequent HFA antibodies were Ch and Yta specificities (36.7%). In 9 patients additional alloantibodies were present. Of those 30 patients 9 patients were transfused; 7 with homologous red blood cells (RBCs) and 2 with autologous RBCs. A total of 46 compatible homologous RBCs were transfused. In 2 patients with multiple antibodies; one with anti-Yta, -Jkb, -E and anti-Cw and other with anti-Ch, -K and anti-Cw 16 units of incompatible RBCs to HFAs were transfused. In 2 pregnant women with HFA antibodies intrauterine transfusions were performed. None of the units were supplied internationally.
Summary/Conclusions: Antibodies to HFAs are a transfusion hazard, as compatible blood is often very difficult to obtain. It may be possible to find an antigen-negative blood for transfusion from a Rare Blood Donor Panel or a Blood Bank of Frozen Blood or to search for compatible RBC between siblings and close relatives. Also, an autologous transfusion program, erythropoietin and iron support, could be provided. In patients with HFA antibodies other clinically significant alloantibodies represent an additional transfusion risk because they are extremely difficult to detect and antigen negative blood is even more difficult to obtain.

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IDENTIFICATION OF 'STORED RED CELL ANTIBODIES' IN THE PLASMA OF FOUR PATIENTS REFERRED TO THE RCI LABORATORY AT THE IBTS

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Background: Red cell antibodies specific to stored red cells but not fresh red cells have been reported. We report the detection of apparent 'stored red cell antibodies' in the plasma of four patients recently referred to the Red Cell Immunohaematology (RCI) Laboratory at the Irish Blood Transfusion Service (IBTS).

Aims: To investigate the hypothesis that the reactivity detected in these samples was due to a 'stored red cell antibody'.

Methods: Antibody investigation was initially performed by gel column technique using Bio-Rad ID panels. The samples were also tested against various red cell panels from other manufacturers (NHSBT, Quotient, Grifols and Immucor) and using various techniques including pre-warmed tube IgG, LISS 18C tube and allo-adsorption. Direct antiglobulin test (DAT) was performed on Bio-Rad ID gel cards. Cross-matching was performed using saline room temperature & IAT techniques on Bio-Rad ID gel cards.

The patients' plasma samples were tested for successive weeks against segments from two fresh donor red cells (suspended in SAGM) and against cells from the donor units maintained in saline.

Results: Reactivity with the majority of panel cells was seen with all four samples. In addition, the auto-control was positive in IgG +/- C3d for 3 samples. Pan-reactivity was detected by LISS 18C tube technique. Allo-adsorption, performed on 2 out of 4 samples, did not remove reactivity. Pre-warmed tube techniques were pan-reactive (samples 1 & 2); with some reactivity remaining for samples 3 & 4.

All four samples initially showed compatibility versus fresh donor units (bled ≤7 days previously). Incompatibility was detected versus the donor cells stored in SAGM by week 6 (sample 2) and week 8 (samples 1, 3 & 4). The age of the donor cells was 37, 52, 54 and 58 days respectively from date bled. Incompatibility was detected versus the donor cells stored in saline by week 4 (sample 2), week 5 (sample 3) and week 6 (samples 1 & 4). The age of the donor cells were 25, 33, 39 and 43 days respectively from date bled.

DAT on the donor unit red cell suspensions was negative at all times. An inert patient sample was also cross-matched against the donor unit segments: the inert control was unreactive on all dates of testing.

Summary/Conclusions: Four patients were found to have antibodies to stored red cells that reacted against significant numbers of reagent red cell panel cells, but did not react against fresh donor red cells. This reactivity may be due to binding of antibody to surface antigens that are exposed as red cells age. Stored red cell antibodies may show variable reactivity versus reagent red cell panels that comprise fresh and/or frozen reconstituted cells. Antibodies to stored red cells can cause delays in identifying suitable donor units for cross-matching, as various avenues are examined to explain the anomalous results, assign specificity to the reactivity and exclude the presence of allo-antibodies.

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Abstract has been withdrawn.

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RED CELL ALLOIMMUNIZATION AMONG TRANSFUSION-DEPENDENT THALASSAEMIA PATIENTS IN ISLAMABAD, PAKISTAN

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Background: Beta thalassaemia major is an inborn haemolytic genetic disorder originated as a result of abnormal production of β-globin chain. It appears in early age in 95% of the patients and is regarded as the most prevailing heterozygous autosomal disorder across the globe with the birth rate of about 300,000 per annum. Beta thalassaemia exists in almost 5% inhabitants and nearly 5,250 β-thalassaemia major carriers are born every year in Pakistan. The proposed management for beta thalassaemia major is regular blood transfusion every 3–4 weeks to maintain the Hb level from 9 to 11.5 g/dL. Other mode of cure of thalassaemia is the bone marrow transplantation, which is out of reach of most of them. This regular blood transfusion may be associated with development of red cell alloimmunization.

Aims: The current study assessed the rate and specificity of red cell alloantibodies in beta thalassaemia major patients and to find the variables affecting the extent of alloimmunization as a result of repeated blood transfusions.

Methods: A prospective analysis of 475 thalassaemia major patients, who had already received more than 10 transfusions, was conducted at the Thalassaemia Centre of Shaheed Zulfiqar Ali Bhutto Medical University, Islamabad, Pakistan. Blood samples were collected in plain anticoagulant free tubes. Blood grouping of all patients was performed initially. After blood clotting, serum was separated through centrifugation at 1,500 g for 10 min. Serum was then analyzed for detection of new antibodies to RBC antigens. Commercially prepared DiaCell I+II+III reagents (DiaMed, Switzerland) were used for antibody screening through standard tube method. After antibody screening, positive samples were further processed through 11-cell panel for antibody identification. Antibody specificity was determined and confirmed through both DiaPanel and ID-DiaPanel, using manual tube method and gel card technology respectively.

Results: In a total of 475 cases studied, 77 (16.2%) were detected with 82 alloantibodies against red cell antigens. Among these 77 patients, double alloantibodies were found in 5 (6.5%) patients. Most frequently occurring antibodies were of Rh blood group system followed by Kell system. Anti-D and Anti-E had highest incidence and were found in 24 and 23 patients respectively. Anti-K antibody was observed in 11 and Anti-c in 3 patients. Anti-C, Anti-e and Anti kpa were found in 2 patients individually. Similarly Cw, k, Jka, Anti-Fyb and Anti-s antibodies were detected in one patient each. About 64.94% (50/77) cases of alloimmunization were found in 11–28 years of age groups. More alloantibodies were detected in males, splenectomized patients, in those who initiated their transfusions before the age of 2, in patients receiving non leukoreduced blood and in B and O blood group patients.

Summary/Conclusions: The study showed significant frequency of alloantibodies production in multi-transfused subjects. To avoid the hazardous effects of alloimmunization in thalassaemia patients, cross matching must be performed at least for Rh and Kell systems as majority of antigens reported in our study are of Rhesus and Kell blood group system. Regular antibody screening and identification will also reduce the risk of alloimmunization.

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ALLOIMMUNIZATION AMONG BLOOD TRANSFUSION-DEPENDENT EGYPTIAN THALASSEMIC PATIENTS

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Background: Thalassemia is the most common genetic disorder worldwide. In Egypt, beta (β) thalassemia represents a major health problem. The carrier rate varies between 5.5 and ≥ 9%; it was estimated that 1,000/1.5 million per year live births have β-thalassemia.

The development of RBCs antibodies remains a major problem in thalassemia major patients who receive regular blood transfusion as a treatment.

Aims: To study the frequency RBCs alloimmunization and autoimmunization among multiple transfused thalassemic patients and to analyze the factors affecting the development of these antibodies.

Methods: Study was carried out on 67 multi-transfused patients with β-thalassemia registered at the NBTC. Patients' records examined for age of patients, age at initiation of transfusion, transfusion interval and status of Splenectomy. Antibody screening and identification was done using 3 cells and 11 cells panel (Ortho Clinical Diagnostics, USA) respectively. To detect auto-antibodies, auto control was carried out using polyspecific cards.

Results: Of the 67 patients, 48 (71.1%) males and 19 (28.9%) females. 56 (83.5%) were RhD positive and 11 (16.5%) were RhD negative. regarding to the spleen state 3 (4.5%) were splenectomized, whereas 64 (95.5%) were not. Among Rh(D) positive patients, 13 were A, 20 were B, 19 were O and 4 were AB. Among Rh (D) negative patients, 4 were A, 4 were B, 2 were O and 1 were AB.

According to age of distribution; patients divided into 5 age groups. In between 1 and 9 years of age total patients were 19 and among them 6 cases of allo-immunization were detected (31.5%). In 10– 19 years of age total 10 patients had alloantibodies among 25 patients (40%). In 20–29 years of age total patients were 13 and

among them 11 had alloantibodies (84.6%). In 30– 39 years of age among 7 patients 6 had alloimmunization (85.7%). In 40– 49 years of age among 3 patients 3 had alloimmunization (100%).).
The rates of alloimmunization among males and females were 56.25% (27/48) and 52.63% (10/19) respectively. P value was more than 0.05, no significant differences observed in rate of alloimmunization with gender and splenectomy.
A total of 37 patients out of total 67 patients (55.22%) developed alloantibodies and 6 (8.95%) developed autoantibodies; 4 of them developed alloantibodies also. Of these patients 15 (41%) developed one alloantibody, 13 (35%) developed 2 alloantibodies and 8 (24%) developed more than 2 alloantibodies. Age at first transfusion was significantly higher in alloimmunized than non-immunized patients (P = 0.042). Out of 11 alloantibodies, Alloantibody against E had the highest incidence (25.37%) followed by K (16.41%), C (13.4%), c (13.4%), D (8.9%), S (8.9%), Fya (5.8%), Jka (4.3%), Lea (2.8%),N (1.49%) and Jkb (1.49%) respectively.
The majority of patients were previously transfused with blood matched only for ABO&D.
Summary/Conclusions: This study re-emphasizes the need for RBC antigen typing before first transfusion and issue of antigen matched blood (at least for Rh and K antigens). Early institution of transfusion therapy after diagnosis is another means of decreasing allo-immunization.

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RED BLOOD CELL ALLOIMMUNIZATION AND RELATED FACTORS IN THALASSEMIA PATIENTS IN SHIRAZ, IRAN

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Background: This study was undertaken to gain an estimation of both the frequency and causes of immunization to red blood cells (RBCs) in transfusion dependent thalassemia (TDT) patients.
Aims: We aimed to evaluate the frequency and causes of immunization to RBCs in TDT patients to facilitate future developments in the treatment of these patients, and to determine the limitation of current practices so that specific measures could be implemented that would lead to a reduction in the incidence rates.
Methods: This cross -sectional study was conducted on 951 (TDT) patients between 20th November 2015 to November 2016 in Dastgheib hospital in Shiraz, Iran. Direct Coombs test, antibody screening,and antibody identification were performed for every TDT patient. Then, the frequency and type of antibodies were surveyed. The clinical and laboratory data of immunized and nonimmunized patients were compared.
Results: RBC immunization was found in 107 patients (11.25%).Indirect agglutination test (IAT) was positive in 62 patients (57.9%) while direct Coombs tests (DAT) was positive in 37 ones (34.6%) of the immunized patients. In addition, 8 patients (7.5%) showed positive results for both DAT and IAT. Alloimmunization rate was detected in 70 patients out of 951 TDT ones (7.4%). The most frequent antibodies were against Kell (61.4%), E (24.3%),and D (18.6%). The results indicated that gender, age of starting transfusion, and splenectomy did not have any impact on the immunization rate.
Summary/Conclusions: In this study, the alloimmunization rate was 7.4%. Antibodies against Kell and Rh and system were found to be the most frequent antibodies. The findings indicate that extended RBC matching, particulary for (ABO/Rh/Kell antigens),must be performed for thalassemia patients and then the effects on RBC immunization rate should be surveyed.

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A META-ANALYSIS OF RED BLOOD CELL ALLOIMMUNIZATION IN IRANIAN BETA-THALASSEMIA PATIENTS

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Background: For many years, transfusion therapy has prolonged the lives of thalassemia patients. But, one of the major challenges of this conventional therapy is alloimmunization. Red blood cell alloimmunization is associated with hemolytic reactions and severe clinical adverse effects. The high rate of alloimmunization in multitransfused patients such as thalassemia always give rise to major burdens both for patients and health services. This meta-analysis study was conducted to determine the prevalence of alloimmunization in Iranian thalassemia patients.
Aims: Nevertheless there are many useful research articles regarding alloimmunization among thalassemia patients, we found a considerable insufficiency about systematic reviews in this field. We performed this meta-analysis to collect some information may be helpful for prevention actions.
Methods: This study was directed based on the computerized literature database. English and non-English articles were searched from 1994 to 2015. Alloimmunization rates and 95% CI calculated by random-effect model. Statistical analysis was performed using Stata 11.2, and ArcGIS 10.3 was used for map construction.
Results: 18 papers from 297 studies involving 4677 patients met our inclusion criteria from literatures. The prevalence of alloimmunization in Iranian thalassemia patients was 10% (CI 95%: 7–13). The most prevalent alloantibody was anti-K (37%), followed by anti-D (29%) and anti-E (20%) in our country.
Summary/Conclusions: The prevalence of alloimmunization among Iranian thalassemia has not been decreased from 1994 to 2013. Despite detecting D antigen in pretransfusion test, the rate of anti-D is high in our population. It shows the importance of more investigation on data on D variants. Although the rate of alloimmunization is low in Iran compared with many other countries, there is a need to adopt some strategies in order to reduce alloimmunization. Hence, it can be inferred that screening programs, which find the donors with the same phenotype of RBC specific antigens, may be helpful to make a pool of donors with the same expression of the most immunogenic RBC antigens such as Kell and Rh other than D antigen.

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PREVALENCE AND SPECIFICITY OF IRREGULAR ANTIBODIES AMONG MULTIPLE TRANSFUSED PATIENTS IN XINYANG CITY

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Background: We found that alloimmunization in long-term transfused patient was a major cause of incompatible cross-matching. But due to the cost and limitation of our laboratory conditions, antigen-matching cannot always be performed.
Aims: To find out the prevalence and specificity of irregular antibodies among multiple transfused patients in Xinyang city.
Methods: The serum from 81 patients with more than 5 transfusions were submitted to irregular antibody screening by micro column gel technology. If the result was positive, the sample was further performed to the identification of alloantibodies.
Results: 81 individuals were enrolled in this study, and among them, 36 patients were administered 5 transfusions, 31 were transfused for 6 to 10 times, and 14 were transfused for more than 10 times. 17(20.99%) cases were detected positive, and the positive ratios for the three groups were 11.11%, 22.58%, and 42.86%, respectively. Second antibodies were founded in two patients after they received 8 or 13 transfusions. The most frequently indentified alloantibody was anti-E (6/19), followed by anti-Le^a (4/19), anti-M(4/19), anti-Jk^a(2/19), anti-c(2/19), anti-C(1/19). Anti-Le^a was at higher frequencies in our city compared to many regions of China, and the distribution of other antibodies was similar to the regions of Yangtze River Basin, but different from the northern regions of Henan Province.
Summary/Conclusions: The distribution of irregular antibodies in multiple transfused patients of Xinyang was different from those in other regions, and although the sample size was small, but it may be also helpful to formulate strategies for these special patients and guide transfusion practice.

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FREQUENCY AND SPECIFICITY OF RED BLOOD CELL ALLOANTIBODY OF TRANSFUSED PATIENTS IN TAIWAN

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Background: Pretransfusion test including ABO typing, Rh test and antibody screening is very important to prevent the transfusion reaction of the inconformity between donor's red cells and recipient's immune-mediated hemolytic blood transfusion reaction. Two major types of irregular RBC cell antibodies: alloantibodies and

autoantibodies are considered potentially clinical significant associated with transfusion reaction and hemolytic diseases.
Aims: The antibody screening test operated in blood bank and detected the presence of alloantibodies. This study is to analyze and investigate the RBC alloantibodies distribution in Chinese population.
Methods: Blood samples from 2006–2016 were under detection for RBC irregular antibodies screening and identification in Taichung Tzu Chi Hospital, Buddhist Tzu Chi Medical Foundation, Taiwan. All blood bank samples were screened by manual polybrene method or classical 3 phases method, and further alloantibody identified by the RBC panel cells (Sanquin, Netherlands). Patients' transfusion history, age, gender, alloimmunization, and transfusion reaction were retrieved from hospital computerized blood bank records.
Results: Total of 30,054 patients were included in this study from 1997–2016 and 935 patients were identified as irregular antibody positive. Total of 1,175 alloantibodies were identified on those patients. The average positive rate of alloantibody was 3.1%. Total of 411 Male patients (44%) and 524 female (56%) in alloantibody positive population. The detection rate of positive alloantibody is no significantly differences between male and female in our study. In the alloantibody, the major specific antibodies are Anti-Mia (34.2%), Anti-E (28.1%) and Anti-c (10.3%).
Summary/Conclusions: Depending on different groups of patients or race populations, and the sensitivity of screening methods, the alloantibodies were found in 0.3%~38% of the population. In this study, the alloantibodies rate is approximately 3.1%. Alloantibodies may result from pregancy, transfusion, drugs, or even transplantation. In the clinical practice, once the alloantibody has been identified, the antigen-negative RBC or blood products must be selected for the further transfusions. Antibodies which reacts at 37°C or patient with IAT are clinical significants. The blood bank should provide the blood products which is negative for corresponding to RBC antigen. However, matching for the suitable blood products could be time consuming, labor intensive and even costly especially for the rare antibodies or multiple antibodies patients. In order to prevent those unwanted complications, most blood banks provide compatible blood as far as possible. To recognize and understand the frequencies and distributions of alloantibodies thoroughly is very important to improve the transfusion safety.

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RISK OF RH ALLOIMMUNIZATION IN SICKLE CELL DISEASE PATIENTS WITH RHCE VARIANTS

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Background: RhCE variants are common in Sickle Cell Disease (SCD) patients and can lead to partial antigens or the lack of high frequency antigens, such as hr^B and hr^S. Patients with those phenotypes may be involved in alloimmunization and delayed hemolytic transfusion reactions but the real risk is still unclear.
Aims: Based on this, we identified SCD patients with RhCE variants previously transfused and performed a retrospective study investigating the risk of Rh alloimmunization after transfusion of incompatible red blood cells (RBC).
Methods: SCD patients with at least six-year transfusion history were genotyped for the presence of *RHCE* altered alleles. *RHCE* genotyping was performed in 237 patients using PCR-RFLP to 48G>C, 254C>G and 1025C>T changes, SSP-PCR to 1006G>T and Exon 5 sequencing. *RHCE* genotypes with altered alleles and predicted phenotypes were determined.
Results: From 237 patients, 32 (13.5%) presented altered *RHCE* alleles predicting 16 partial e antigen, 9 partial c antigen, 3 partial e with partial c antigens and 4 h^B- phenotypes. Among these, 25 were transfused in our Institution, including 3 patients transfused with more than 50 RBC units, of which 1 with partial c and 2 with partial e. Four patients received more than 10 RBC units, 3 of them with partial e and 1 with partial e and hr^B-. When we analyzed the RBC alloimmunization in those patients, we observed that no patient developed Rh alloantibodies in response to the RhCE variants. RBC antibodies with other specificities were observed in 5 patients, including one patient with partial e who received 51 e-positive RBC units but had developed only anti-D, -C, and -S.
Summary/Conclusions: Our results showed that 13.5% of the SCD patients studied had *RHCE* variant alleles predicting partial c, partial e and hr^B- phenotype. Although they have received blood with conventional RhCE antigens, no patient developed alloantibodies, even those alloimmunized to other antigens. Further studies are necessary to look for more clinical and laboratory evidences in order to determine the relevance of variant *RHCE* alleles predicting partial antigens in SCD patients.

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Abstract has been withdrawn.

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Abstract has been withdrawn.

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Abstract has been withdrawn.

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PREVENTION AND MANAGEMENT OF RHD-CE ALLOIMMUNIZATION IN SICKLE CELL DISEASE (SCD): MOLECULAR CHARACTERIZATION IN A COHORT OF AFRICAN PEDIATRIC PATIENTS IN NORTHERN ITALY

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Background: Blood transfusion is a cornerstone in the management of sickle cell disease (SCD). The delayed hemolytic transfusion reaction is frequent and represents a life-threatening event. The main cause of post-transfusion complications is alloimmunization due to the different distribution of red cell antigens between donors, most of them Caucasian in Northern Italy, and patients with SCD, who are primarily of African ancestry.
Aims: The first goal of this study was to characterize variant *RH* alleles in children and adolescents with SCD to identify patients with high risk of alloimmunization. A second goal was to create a dedicated pool of African blood donors to support antigen-matched blood transfusion and reduce the risk of alloimmunization.
Methods: *RH* genotypes were determined in DNA samples from 59 SCD patients. RHD and RHCE BeadChip™ kits (Immucor/BioArray Solutions) were used to identify the *RH* variants. The RHD BeadChip™ kit can detect up to 35 mutations and provide determinations on at least 75 RhD variants, while the RHCE BeadChip™ kit detects up to 25 mutations, thus allowing to determine at least 35 RhCE variants. The same samples were also tested with the HEA BeadChip™ kit, which is able to provide molecular characterization of 38 erythrocyte antigens and phenotypic variants. Serological extended characterization was performed in all patients (hemoagglutination in tubes, Bio-Rad).
Results: The median age of SCD patients was 10.71 years. The most frequent Countries of origin of our population were Ghana and Nigeria (76.27%). We identified variant *RHD* alleles in 23/59 patients (39%), with 6 of them showing *RHDΨ*. One patient (1.70%) presented a deletion of the RHD gene. *RHCE* variant alleles were identified in 51/59 cases (86.44%). Only in one patient (1.7%) the *RHCE* genotype was not clearly identifiable. As expected, the most frequent alleles identified in our population were *RHCE*ce48C*, *RHCE*ce733G* and *RHCE*ce48C,733G,1006T*. Twenty nine patients (49.15%) had *RHCE* genotype with homozygous or heterozygous alleles predicting for partial antigens. Nucleotide sequence analysis is ongoing on the samples in which allelic variants were not clearly identifiable with the methods we used. With the help of local associations, we have been involved in a campaign of recruitment of new African donors, with the aim of creating a dedicated donor pool for these patients.
Summary/Conclusions: The number of SCD patients dramatically increased in the last decade in Northern Italy due to migration fluxes from Sub-Saharan African countries. RhD-CE variants are very frequent in these patients and can be clinically significant. It is important to establish programs for *RHD-CE* genotyping so that a better match between SCD patients and donors can be achieved in order to reduce the risk of alloimmunization and post transfusion hemolytic reactions.

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ASSOCIATION OF THE TT GENOTYPE AT SNP RS2236379 IN THE PRKCQ GENE WITH ANTI-E ALLOIMMUNIZATION

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Background: Studies with patients with sickle cell disease (SCD) and other haematological diseases have been conducted to identify factors that may be associated with increased susceptibility to red blood cell (RBC) alloimmunization. These factors include gender, age, number of transfusions, inflammatory status, history of autoimmunity, ethnic differences between donors and transfusion recipients, as well as genetic markers that can modulate the alloimmunization process.
Aims: In this study, we investigated specific polymorphisms (SNPs) in the GZMB, PRKCQ and RHAG immune response genes in patients with SCD and beta thalassemia who receive regular blood transfusions in order to identify possible genetic markers of RBC alloimmunization.
Methods: Genotyping was performed by PCR-RFLP for the SNPs: RHAG G280R (rs104893987) RHAG F67L (rs11635654), RHAG I369I (rs141568382), GZMB (rs564414889), GZMB (rs8192917) and PRKCQ (rs2236379) in DNA samples of 126 patients with SCD, of whom 56 were alloimmunized to RBC antigens and 70 were non-alloimmunized, and in 52 DNA samples from patients with beta thalassemia, 20 of whom were alloimmunized to RBC antigens and 32 were non-alloimmunized. The same SNPs were analyzed in 171 samples from blood donors. The frequency comparison was performed through the SNPStats platform of the Catalan Institute of Oncology (ICO), University of Barcelona. The significance level adopted for the tests was 95% ($P \leq 0.05$).
Results: Most alloimmunized patients had anti-Rh antibodies, mainly anti-E, which was found alone or in combination with other antibodies. Among the polymorphisms analyzed, we found a significant association of the rs2236379 SNP TT genotype in the kinase theta protein gene (PRKCQ rs2236379) with anti-E antibody alloimmunization in these patients. The results presented statistical significance in the codominance, recessive and additive models. Other SNPs and factors analyzed such as gender and number of transfusions did not present statistically significant results.
Summary/Conclusions: Considering the high frequency of anti-E antibodies in patients receiving chronic transfusions, the results obtained with this study may contribute in the future to the prior identification of patients who are at higher risk of anti-E formation and who should therefore receive Rh matching blood to prevent alloimmunization and delayed haemolytic transfusion reactions.

P-450

PREDICTING ANTI-RHD TITERS IN DONORS: BOOSTERING RESPONSE AND DECLINE RATES ARE PERSONAL

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Background: Anti-RhD immunized donors provide anti-RhD immunoglobulins used for the prevention of rhesus disease. These donors are periodically hyper-immunized (boostered) to retain a high titer level of anti-RhD.
Aims: To try and model the RhD antibody response in actively immunized donors in order to optimize donor boostering strategies.
Methods: We analyzed anti-RhD donor records from 1998 to 2016, consisting of 30,116 anti-RhD titers from 755 donors, encompassing 3,372 booster events. Various models were fit to these data to allow describing the anti-RhD titers over time.
Results: A random effects model with a log-linear anti-RhD titer decline over time and a saturating titer response to boostering is shown to fit the data well. This model contains two parameters characterizing the individual donor and two general model parameters. The average individual log2 decline is 0.55 per year, i.e. a 32% decline in absolute titer, with half of the donors declining between 13% and 41% per year. Their anti-RhD titer peaks around 26 days following a booster event. Boostering response reduces with higher titers at boostering; at median titer (log2 11) the mean increase per booster is log2 0.38, i.e. from an absolute titer of 2048 to 2665 (+30%), with half of all donors increasing between 16% and 65%.
Summary/Conclusions:: The model describes anti-RhD titer change per individual with only four parameters, two of which are donor specific. This information can be used to enhance the blood bank's immunisation programme, by deriving individualized immunization policies in which boostering is adjusted to the anticipated anti-RhD decline, effectiveness of boostering and titer levels required.

P-451

CLINICAL BENEFITS OF RH GENOTYPE MATCHING IN PATIENTS WITH SICKLE CELL DISEASE

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Background: Knowledge of the prevalence of RH variants supports development of strategies to match RH to avoid Rh alloimmunization and the risk of hemolytic transfusion reactions and/or poor transfusion outcomes. Molecular Rh typing has been performed to identify the *RH* altered alleles and is playing an important role in expanding matching of SCD patients and donors in the RH system.
Aims: In this study, we characterized variant *RH* alleles in patients with SCD and in African Brazilian donors, in order to support RH-matched blood for transfusion and investigated the clinical outcomes.
Methods: RH genotypes were determined in 161 DNA samples from SCD patients and 288 DNA samples from African Brazilian donors. Laboratory developed tests (LDTs), RHD BeadChip™, RHCE BeadChip™, cloning and sequencing were used to determine *RHD-CE* genotypes among patients and African Brazilian blood donors. In order to find compatible donors for SCD patients with RH variants alloimmunized to Rh antigens, RH genotype matching was performed. We considered the total of red blood cell units requested for each patient and a number of 2 donations per year for the compatible donors.
Results: We found different combinations of RH variant alleles in 48 polytransfused patients (29.8%), predicting partial antigens or lack of high prevalence antigens such as hr^B and hr^S. Twenty-three patients (47.9%) were alloimmunized to Rh antigens. The most common *RHD* variants found on those patients were *RHD*DAR, RHD*weak D type 4.0, RHD*DIIIa, RHD*DAU0* and *RHD*DAU4*. And the most common *RHCE* variants in this population of patients were *RHCE*(C)ce^{S,} RHCE*ce48C, RHCE*-ce733G, RHCE*ceAR, RHCE*ceEK* and *RHCE*ceMO*. RH genotype matching performed on 12 alloimmunized patients improved their clinical outcomes as shown by the increase in their Hb levels and reduction in their percentage of HbS, better *in vivo* RBC survival and diminished frequency of transfusions.
Summary/Conclusions: The provision of RH genotyped matched units may decrease the rate of Rh alloimmunization and delayed hemolytic transfusion reactions in patients with SCD. Knowledge of clinically relevant *RH* genotypes in patients with SCD and in donors is essential to assessing the potential risk of alloimmunization and is necessary to determine the number of donors required to support patients with SCD with RH genotype matched units.

P-452

MULTIPLEX BLOOD GROUP TYPING WITH CELLULAR SURFACE PLASMON RESONANCE IMAGING

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Background: Surface plasmon resonance (SPR) is a widely used label-free method to follow molecular interactions in real time. Recent advances in SPR based cellular measurements has provided evidence that interactions between molecules on SPR-sensors and various cells can be monitored. Binding of red blood cells (RBCs) to blood group antigen specific antibodies in SPR has been already demonstrated by various groups, but so far no methods have achieved implementation of an economical SPR-platform capable of typing multiple blood groups simultaneously.
Aims: Our aim in this study was to provide a proof-of-concept for multiplex blood group typing with SPR. To evaluate the performance of our platform we compared our blood typing results from a blind typing of over 900 donor samples with results obtained by National Screening-laboratorium Sanquin, Amsterdam (NSS).
Methods: Single blood group specific monoclonal antibodies (anti-A, anti-B, anti-D, anti-C, anti-c, anti-E, anti-e and anti-K) were covalently spotted onto SPR sensors through amine-coupling in duplicates. Sensor was generated by Continuous Flow Microspotter (CFM) using the Wasatch Microfluidics platform and SPR measurements were performed using the IBIS MX96 platform. First test samples of known blood group type were measured to validate each spot on each SPR sensor before testing donor samples. RBC samples were incubated first without flow for three minutes over the sensor in the flow chamber, allowing the gravitational sedimentation of RBC onto this sensor. This sedimentation step allows the close interaction of RBCs with the sensor, and is impossible on most other SPR equipment, which has the senor optics reversed, which is incompatible with gravitational sedimentation. After

the sedimentation, a buffer flow of 20 µl/s was applied for 50 seconds, washing away RBC not interacting with sensors, only retaining RBCs specifically bound by antibodies. We characterized RBC binding on each antibody spot by comparing measured response at the end of the flow (T) and sedimentation (S) phase by the T/S ratio (Schasfoort, 2013, Anal. Biochem.). Cutoff values were set as the mean plus four SEM of the negative test samples for each antibody. After each sample the sensor was regenerated with 0.1% Triton X-100.
Results: Our results show that each sensor can be used for at least 96 measurements. Although the calculated T/S ratio might change over the measurement cycles, in most cases – depending on the antibody – the difference remains sufficient to provide accurate typing. Accordingly, our results for over 900 samples were in a 100% agreement with routine blood group typing at NSS for A, B, D, C, c and K. However, some discrepancies were found for the less well characterized antibodies in our series (anti-E (681/6) and anti-e (857/78)).
Summary/Conclusions: Altogether, our results suggest that depending on the antibody used, SPR can be used reliably for blood group typing, in this case for up to 96 different measurement in a single run.

P-453

DEVELOPMENT AND APPLICATION OF ETERNAL BLOOD GROUP ANTIGENS INDEPENDENT OF LIVING RED CELLS

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Background: Red cell membrane harbors blood group antigens, and cell membrane antigen depending on living cells must disappear simultaneously with the hemolysis. It is hard to imagine that red cell compatibility tests could have being done clinical-routinely without intact and living red cell reagents. Up to now, innumerable efforts to delay expire of the living cell antigens have not got satisfactory solution. It is a few weeks to expire for red cell reagents in 4°C; might be a few years in liquid nitrogen, but with very poor recovery cells after thawing and too high cost.
Aims: To develop and evaluate a novel method for conserving blood group antigens.
Methods: Magnetized Erythrocyte Group Antigens (MEGA) was developed by our research group by replacing the content of erythrocytes with nano-magnetic beads. Purified ABO/RhD soluble antigens from expired RBC have been made as reagents and been used routinely in forward/reverse ABO grouping and RhD grouping simultaneously in Solid-Phase Red Cell Adherence Assay (SPRCA). Synthesized peptides with Mur and/or Hil antigen specificities, which have clinical significance in some ethnic people of Asia and American Indian, have been used for antibody detections with novel Chromatography Fluorescence Immunoassay and ELISA.
Results: The results showed that rather strong ABO/RhD, Mur and Hil antigenicities were kept on the MEGA.
Summary/Conclusions: On MEGA membranes wherein nearly intact and many of group antigens originate from red cell membranes with clinical significance, such as ABO, RH, MNS, KEL, et al., remain easily detectable when stored in 4°C for over five years at least. The intrinsic applications of MEGA should be (1) as standard ABO antigens for testing high titre ABO antibodies in blood products; (2) to conserve the rare group antigens eternally; (3) to demonstrate blood group antibody specificity; (4) to establish criteria for red cell blood group antibody reagents; (5) to purify blood group antibody with ads/elu method, and so on.

P-454

RAPID EXTENDED PHENOTYPING OF DUFFY, KIDD AND SS ANTIGENS USING MDMULTICARD LATERAL FLOW TECHNIQUE

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Background: Antibodies directed against Fya, Fyb, Jka, Jkb, S and s antigens are capable of causing transfusion reactions or haemolytic disease of fetus and newborns (HDFN). Performing extended phenotype matching (extending to Duffy, Kidd and Ss) beyond standard matching (ABO/Rh phenotype) allows clinical benefit to multi-transfused patients preventing approximately 70% of alloimmunizations. A rapid typing of rare antigens is useful for finding antigen-negative units in life-threatening emergency situations as well as facilitating transfusion of extended phenotyped-match blood to patients with a reactive DAT. A lateral flow assay allows a simultaneous and rapid typing of Duffy, Kidd and Ss antigens, with the detection of Fy(b) weak antigen and mixed-field reactions.
Aims: Evaluate sensitivity of the MDmulticard® Basic Extended Phenotype, especially focusing on the ability to correctly type rare phenotype in DAT+ patients and identify weak antigen expression.
Methods: 30 EDTA anticoagulated samples were tested: 10 donors, 4 DAT+ patients, 6 newborns, 8 hospitalized patients, 2 multitransfused. Extended serological typing with MDmulticard® Basic Extended Phenotype (Medion Grifols Diagnostics, Dudingen, Switzerland) was performed according to manufacturer' protocol: 100 ml of diluted whole blood were transferred to the application zone of the MDmulticard, followed by a double pipetting of 300 ml of a rinsing solution. Results were interpretable after 5 min: positive results as distinct red bands, whereas negative results lacking the respective bands; results were evaluable if the process control spot (val) and the autocontrol spot (ctl) were positive and negative respectively. Results obtained from MDmulticard were compared with phenotype (Immucor, Germany) and/or genotype (BLOODchip ID, Progenika-Grifols, Spain) when serological typing was not reliable (transfused or DAT+ patients).
Results: All results of the donor and patient samples were full concordant with the serological/molecular typing. Three samples with genotypic Fy^x positivity (confirmed by adsorption/elution test) were correctly recognized as Fyb positive by MDmulticard technique, while serology typed them false-negatively. Agglutination mixed-fields due to previous transfusions were identified as a positive band weaker than patient own red blood cells. Reactive DAT samples were correctly typed (autocontrol being always negative), avoiding molecular typing. We performed the test starting from whole blood for all samples, but we observed that in samples with Hct < 30% Jkb positive band may be weaker than expected, suggesting a preferable use of erythrocyte sediment in these cases. Results from newborn samples, often insufficient, clotted or haemolytic, were clear.
Summary/Conclusions: MDmulticard is a fast and simple to use technique for rapid simultaneous typing of rare erythrocyte antigens in routine and emergency transfusion setting. It is accurate as conventional serological technique, without the interferences of IgG autoantibodies which cause false positive reactions with IAT serology methods. The high sensitivity of the lateral flow technology allow to identify Fy(b) weak antigen expression and mixed-field reactions, contributing to solve complex cases. The simplify standard method and its manageability can be applied by non-experienced staff obtaining reliable extended phenotype results to improve transfusion safety. It is to be hoped that the technique will be implemented with interconnection to LIS and traceability of the results.

P-455

SPECIFICITY OF RED BLOOD CELL ALLOANTIBODIES DETECTED IN A 12-MONTH PERIOD

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Background: Alloantibodies in the patients' serum (due to previous red blood cell transfusion or pregnancy) is the most common cause of incompatibility during the pre-transfusion testing. The anti-D specificity predominated in the past, but this is no more the case, due to immunoprophylaxis of Rhesus negative pregnant women. Alloimmunization against other red cell antigens, depends on the transfusion policy, ethnicity, number of transfusion events, number of units received, age at first transfusion etc.
Aims: The objective of our study was to depict the changing pattern of red cell alloimmunization by analyzing data from a 12-month period.
Methods: Nineteen patients hospitalized from 1/7/2015 to 30/6/2016, as well as 29 patients referred to our laboratory from other hospitals, were screened because of incompatibility problems. Patients with homozygous Thalassemia, Thalassemia Intermedia, Sickle cell disease and autoimmune hemolytic anemia, were excluded from analysis. Alloantibodies were found in 21 males and 27 females. The average age was 69.9 years (range 13 to 90). Twenty nine patients were hospitalized in internal medicine and 19 in surgical departments. A clear history of previous blood transfusion was given by 36/48.
The study protocol required: detailed medical history (medication, previous transfusions pregnancies, etc.), blood group typing, direct Coombs test, extended red cell phenotype in the Kell, Kidd, Duffy, MNS, P, Lewis and Lutheran systems, antibody screening with a commercial 3-red cell panel and antibody identification with an 11-cell panel, by three techniques (Liss-Coombs, enzyme, neutral at 4o C).

Results: A single alloantibody was identified in 14/48 patients, two or more alloantibodies in 24/48 and a natural anti-M alloantibody in 1/48.
In 9 patients with a mixture of multiple antibodies, not all of them were identified.
In the remaining 39 patients, the following 72 alloantibodies were identified:
2 anti-D, 7 anti-E, 1 anti-e, 3 anti-C, 6 anti-c, 4 anti-Cw *(Rhesus)*.
11 anti-Kell, 5 anti Kpa *(Kell)*.
5 anti-Jka, 6 anti-Jkb *(Kidd)*, 6 anti-M, 2 anti-S *(MNS)*, 4 anti-Fya, 3 anti-Fyb (*Duffy*).
4 anti-Lua (*Lutheran*), 3 anti-Lea (*Lewis*).
Red cell units were selected according to the extended phenotype or at least negative to the specific erythrocyte antigens against which the patient had formed antibodies. *Determination of the phenotype was not possible in 5/48 cases: 3 due to recent transfusions and 2 due to positive direct Coombs test.*
Summary/Conclusions: Our policy consists of routinely selecting units matched for Rhesus C, c, E, e and Kell, if available. Many patients in this series had obviously received ABO and Rh D matched-only units, in the past.
Remarkably, only 2/72 alloantibodies showed anti-D specificity.
Specificities in the Rhesus (23), Kell (16) and Kidd (11) systems predominated (clinically significant).
Alloimmunized patients must be protected from further alloimmunization by extended matching for C, c, E, Cw, K, Kpa, Fy(a or b), Jk(a or b), S or s.

P-456

RAPID EXTENDED PHENOTYPING USING LATERAL FLOW TECHNOLOGY

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Background: Extended red cell antigen typing might not only be helpful in identifying the best matched RBC product to reduce alloimmunization in multi transfused patients (e.g. sickle cell anemia), but also in antibody identification in patients with complex red cell alloantibodies and/or panagglutinating autoantibodies (e.g. autoimmune hemolytic anemia).
Aims: The new MDmulticard® Basic Extended Phenotype (Medion Grifols), based on lateral flow technology, allows to perform simultaneous typing for Jka, Jkb, Fya, Fyb, S and s antigens in about 8 min. In this study, we compared the performance of the MDmulticard® with standard laboratory methods and evaluated its usability in daily laboratory routine.
Methods: Phenotyping of unselected samples of blood donors (45) and of patients (23 adults, 5 newborns) was performed using MDmulticard® Basic Extended Phenotype and the results were compared to those of gel column agglutination and solid phase technology, respectively. In addition, 11 DAT positive samples (reactivity of 2+ to 4+) of patients with known autoantibodies and 12 mixed-field samples from patients who had been previously transfused with random (not selected for Kidd, Duffy and Ss antigens) red blood cells were investigated. All DAT positive and mixed-field samples were genotyped after DNA extraction using PCR-SSP.
Results: The results obtained by testing of donor, patient and neonatal specimens with the MDmulticard® Basic Extend Phenotype card were consistent with those obtained by our standard methods in all 73 samples evaluated. This was also the case for the 11 DAT-positive samples. Mixed-field agglutinations were observed in 8 of the 12 tested samples by MDmulticard® and in 4 samples tested with the column agglutination (gel) card, respectively. Furthermore, extended phenotyping by MDmulticard® was found to be very useful in identifying rare phenotypes (e.g. U-negative) and the corresponding antibody specificity.
Summary/Conclusions: MDmulticard® provides an easy and reliable method for extended red cell phenotyping of various blood samples, including those from patients with positive DAT due to autoantibodies. However, further studies are needed to confirm these findings.

P-457

EXTENDED RED CELL PHENOTYPING-IS IT THE RIGHT STEP TOWARDS BLOOD SAFETY??

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Background: Red cell antigen alloimmunisation is the major challenge in current Transfusion practices. The need for implementing routine Rh-Kell phenotyping for all donors and patient screening is being realised all over the world to prevent the risk of alloimmunisation as far as possible. Fortis Escorts Heart Institute in India is a leading hospital in India, specialized in cardiology and other specialities has high input of paediatric and adult patients from all over the world.
With a high number of cardiac surgeries and organ transplant programme running, use of blood components is in accordance. Our patients may need repeated transfusions time, massive transfusions and at times undergo redo surgery having multiple transfusion episodes. So a high risk of alloimmunisation and antibody development exists.
To eliminate the risk of alloimmunisation and ensure safer transfusions in patient's lifetime, our hospital is among the first few centre in India to introduce Rh-kell phenotyping for all patients and blood donors.
Aims: To assess feasibility of doing extended red cell phenotyping and cost effectiveness.
Methods: Study Duration: 58 months. All the patients anticipating blood transfusion and all donors subjected to Rh-kell phenotyping and 3 cell panel antibody screening as a routine.
Total samples tested 37,879:30,209 donors & 7,670 patients (adult and paediatric) on fully automated immunohematology analyser- QWALYS 3 (Diagast, France) using Erythrocyte Magnetized Technology®.
Data of the donor units are saved in the Microsoft Excel sheet with the donation & Unit details. Patient phenotyping and antibody screening (IgM +IgG) is done at the time of admission. For transfusion, ABO & phenotypically matched units are issued after an Immediate spin cross match.
Results:

	A	AB	B	O	D	C	c	E	e	K
Donor Blood Group										
Negative	472	227	684	675	2,057	4,056	12,858	24,293	379	29,551
%	1.56	0.75	2.26	2.23	1.14	2.24	7.10	13.41	0.21	16.31
Positive	6,481	2,700	10,105	8,865	28,150	26,149	17,342	5,906	29,816	647
%	21.45	8.94	33.45	29.35	15.53	14.43	9.57	3.26	16.45	0.36
Patient Blood Group										
Negative	124	48	154	140	470	1,078	3,028	6,113	110	7,518
%	1.62	0.63	2.01	1.83	1.02	2.34	6.57	13.27	0.24	16.32
Positive	1,676	664	2,570	2,294	7,202	6,596	4,653	1,567	7,561	161
%	21.85	8.66	33.51	29.91	15.64	14.32	10.10	3.40	16.42	0.35

Summary/Conclusions: Introduction of Rh–Kell phenotyping of the donors and the patients has improved blood transfusion services drastically. It helped us in many ways:
1. In identifying the phenotype of our population
2. In providing antigen specific red cells to our patients thus ensuring another safety grid by avoiding alloimmunisation
With regards to the high frequency of immunogenic antigens of the Rh-Kell systems, pre-transfusion antibody screening on patients' samples and Rh-Kell phenotyping on donors are essential and needs to be implemented. In long run it is cost effective measure.

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EVALUATION OF A NEW LATERAL FLOW MDMULTICARD BASIC EXTENDED PHENOTYPE® FOR ROUTINE USE IN A TRANSFUSION CENTER

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Background: The MDmulticard® Basic Extended Phenotype (Medion Grifols Diagnostics AG) is intended to be used to quickly perform extended red blood cell phenotypes covering the following clinically significant antigens: Fya, Fyb, Jka, Jkb, S, s. The MDmulticard® Basic Extended Phenotype is based on lateral flow principle including IgM and IgG monoclonal antibodies directed against the mentioned antigens immobilized on a membrane, including internal procedural (val) and negative (neg) controls that confirm the test integrity.
Aims: The aim of our study was to evaluate the performance, reliability, sensitivity and specificity of the MDmulticard® in the real practice of a transfusion center laboratory.
Methods: MDmulticard®, was compared to well-established serological methods, according to a protocol that was designed to simulate the routine workload. A total of 78 samples were tested. Samples from 55 donors were tested with the MDmulticard and with solid phase microplate (Capture-R® Select, Immucor, Norcross GA, USA) as the reference method. Those from 16 segments of donation bags and 7 positive Direct Antiglobulin Test (+DAT) samples, were analyzed with both the MDmulticard and glass beads column (Anti-IgG,-C3d; polyspecific BioVue®, Ortho-Clinical Diagnostics, Raritan NJ) for Fy^a, Fy^b, and s phenotypes and with conventional tube testing with liquid monoclonal IgM antisera (Immucor Gamma®, Immucor, Norcross GA) for Jk^a, Jk^b y S phenotyping. Overall, 468 antigen were therefore assayed (330 from blood donor samples, 96 from segments of donations and 42 from +DAT samples). Concordance between methods was assessed and discrepancies were analyzed. In addition, usability and adaptability characteristics of the card were evaluated.
Results: Concordant results between the MDmulticard® and the reference method were obtained in 456 (97%) of the 468 tested phenotypes. All the results in donor samples were concordant, except for 1+ DAT sample that was invalidated in the reference method (solid phase microplate) due to non-specific agglutination of the negative control, nevertheless the MDmulticard® results for this sample were correct. No discrepancies were observed in the 16 segments of donation bags. In 5 of the 7+ DAT samples, 6 discrepancies were detected; glass bead column agglutination detected the presence of Fy^a antigens in 3 cases, Fy^b in 1 sample and s in another, Jk^a was positive by tube testing in 1 case. However, results from these phenotypes, when tested with MDmulticard®, were negative. We considered these discrepancies as false positive results of the reference method, due to +DAT in indirect antiglobulin test techniques and because of autoagglutination when conventional tube testing was used. Moreover, MDmulticard® testing required very little manipulation, providing results in nine minutes, and it was stable for up to 48 h.
Summary/Conclusions: MDmulticard® Basic Extended Phenotype was an easy procedure that quickly provided information on 6 antigens. This test was at least as reliable as the routine reference method, and adequate to use in acute clinical situations. This assay is an accurate method that allows a correct prediction of the phenotype of clinically relevant red blood cell antigens, as well as allowing the typing of patients with a positive Direct Antiglobulin Test.

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IMPLEMENTATION OF A FULLY AUTOMATED ANALYZER FOR TRANSFUSION MEDICINE ANALYSIS'S, (ORTHO VISION ANALYZER), IN 6 HOSPITALS – EXPERIENCE FROM STOCKHOLM, SWEDEN

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Background: The Transfusion medicine service in Stockholm county, located at six hospitals in Stockholm, performs approximately 90 000 ABO/RhD reverse and antibody screening, 90 000 type and screen, 1 500 cross matches, 90 000 ABO/D confirmation for blood donor, 10 000 phenotypes (Rh, Kell, Duffy, Kidd and MSs), 1 500 antibody panels and 800 titrations. At the four largest hospitals transfusion medicine analysis's of patient samples have been fully automated for a decade using AutoVue, Ortho Clinical Diagnostics(OCD). For blood donors the Erytra from Grifols were used. The two smaller hospitals have worked manually.
This resulted in three different analysis systems, management and processes.
Aims: Our aim was to replace previous automated immunohematology analyzers for patients and donors with new identical instruments and additional facilities. Furthermore all hospitals should be fully automated. Identical instruments entails less quality management (QM), fewer method documents and easier to train and staff. Less reagent verification testing upon arrivals would be required and one service contract with suppliers would be needed.
Methods: ORTHO VISION Analyzer utilizes the Ortho BioVue Column agglutination Technology (CAT) with digital image processing. It has a continuous, random sample access with STAT priority processing. It performs immunohematology routine analysis including antibody titration and reflex test e.g. IAT anti-D of RhD negative donors.
We applied a project working form and organized one team for the implementation process at all six hospitals. The team included project leader, QM, medical and laboratory staff and IT specialists. The installation, training, configuration, maintenance and validation processes were streamlined to provide a short implementation time. The team also introduced new automated functionalities such as antibody titration. Turnaround times (TAT) were collected and analyzed.
Results: All 11 instruments at 6 hospitals were implemented over the pre-decided time frame of 7 months. The instruments were connected to the laboratory information system (LIS) via a network with bidirectional communication. The main implementation problems were IT-related, e.g. the order, results and analysis setups. TATs were unchanged. Every hospital laboratory described a positive experience using Vision.
ABO confirmation of Blood donor samples were optimized for performance during the night shift. The automated antibody titer lead to standardization and time savings and were shown to be comparable to the standard method, i.e. manual titration with ID-card, BIO-RAD.
Summary/Conclusions: The implementation of ORTHO VISION Analyzer at our laboratories was successful. The model with a small implementation team working with overall issues was a success for us and made it possible to have 11 instruments up and running in a short time frame.

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EVALUATION OF THE NEW ERYTRA EFLEXIS® ANALYZER AND DG GEL® SYSTEM FOR ROUTINE USE IN A UK HOSPITAL TRANSFUSION LABORATORY

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Background: The Erytra Eflexis® (Grifols) is a new fully automated, mid-size analyzer that performs pre-transfusion compatibility testing using DG Gel® technology.
Aims: Erytra Eflexis® analyzer performance, usability and adaptability to different workflows was evaluated in the routine environment of a large UK acute hospital transfusion laboratory.
Methods: A comparison study was performed between the Erytra Eflexis® and Erytra (our routine system providing the reference platform). A total of 2,944 tests were performed on 1,214 adult patient samples and 208 donor red cell units. Erytra® Eflexis performance was evaluated according to a series of scenarios designed to simulate routine workload using the system in different configurations. Concordance between systems was assessed and discrepancies analyzed. Time to first result (TTFR), overall turn-around time (TAT) total workload from first result to last result (throughput, results/h), manual "hands-on" time and walk-away time were all recorded.
For ease of use evaluation, we ranked usability features with number of steps and timing of activities including sample sort and loading, routine testing, post-run

procedures, consumables used, and space requirements. Fault recognition and messaging was assessed by simulating failures e.g. reagent absence.
Results: Blood grouping, antibody screening, antibody identification (using panels), direct antiglobulin test, red cell phenotyping and serological crossmatching were successfully tested.
Concordant results between the Erytra Eflexis® Analyzer and reference method were obtained in 99.9% of samples tested. There were 4 discrepancies, all antibody screening (2 false positives, 1 failure to detect a very weak prophylactic anti D and 1 positive reaction not detected on the Erytra but panels on both systems suggested a genuine anti Cw).
TTFR and TAT depended significantly on a number of factors including; number and variety of tests requested and whether the STAT functions were activated.
The analyzer seemed to prioritize antibody screening Prioritization of the group, especially for STAT samples, was considered preferable.
The laboratory team found the software easy to use with some improvements over existing Ertyra software.
Physical design of the analyzer was considered good with easy access to almost all areas. Probe changing was quick and simple.
While the analyzer successfully flagged all error scenarios some messages were considered misleading and could be better phrased.
Summary/Conclusions: Results showed the Erytra Eflexis® offered a robust automated solution for routine transfusion testing. The device could comfortably deal with a medium laboratory (processing 80–100 group and screens per day). It is very flexible being able to deliver grouping, antibody screening and identification, DAT, phenotyping and serological crossmatching,compensating for its' single probe and wash station by clever use of incubators, centrifuges and design features.
This allows a compact design with maximum flexibility without compromising on turnaround times.

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STATUS1 STAT ABO/D + REVERSE AND STAT GROUP CHECK – A NEW STANDARDISED RAPID BLOOD GROUPING TECHNIQUE

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Background: Blood provision in an emergency setting does not allow time for automated methods of ABO/D typing. Manual systems available, including column agglutination (CAT) or tube methods, are either not rapid enough in these scenarios or have no mechanism for automated interpretation or interfacing to LIMS systems. A novel test, STATUS1 STAT ABO/D + reverse and STAT Group Check offer a rapid grouping test suitable for emergency scenarios, with an automated reader capable of interpretation and interfacing to LIMS.
Aims: Compare:
1. ABO/D grouping results between different manual methodologies; STAT ABO/D + reverse, STAT Group Check, tube technique and CAT
2. Time to result for each manual methodology.
Methods: EDTA whole blood samples were tested using each method. Reaction strengths were graded from 0 (negative) to 4+ (strongly positive). 55 samples were tested using the STAT Group Check, tube and CAT techniques. 85 samples tested using the STAT ABO/D + reverse, tube and CAT techniques. The tube technique included monoclonal reagents (Bioscot ABO/D typing) and NHSBT reagent cells (3% in Alsevers) for reverse grouping. Patient red cell suspensions were made using phosphate buffered saline pH7.0.
CAT was performed using: DiaClon ABO/D + Reverse Grouping cards and NHSBT reagent cells (3% in Alsevers) for reverse grouping. DiaClon ABO/Rh for Newborns (Bio-Rad).
Patient red cell suspensions were made using ID-Diluent 2 (Bio-Rad).
STAT ABO/D + reverse included STATUS1 A1 and B cells for reverse grouping, patient red cell suspensions were made using STATUS1 Solution.
Testing time was calculated in minutes, from preparation to result interpretation.
Results: ABO/D typing results were identical in 83/85 (97.7%) of samples tested using the STAT ABO/D + reverse, tube and CAT techniques. One D variant patient, tested D negative in STATUS1 and tube test and positive (3+) in CAT. A weak D patient tested D Negative in STATUS1 and tube tests and positive (1+) in Bio-Rad. Another weak D patient tested weakly positive (1+) in all three techniques. ABO/D typing results were identical in 51/55 (92.7%) of samples tested using the STAT Group check, tube and CAT techniques. Four weak D patients were tested; all tested D negative in STATUS1, all weakly positive (2+) in Bio-Rad and in tube one tested D negative, 3 weakly positive (1+). An A subgroup patient with anti-A1 tested weakly positive (1+) with the A1 cells in the reverse group in all techniques. Total time from preparation to result for each technique was:

- 2 min for STAT Group check tube
- 3 min for STAT ABO/D + reverse
- 4 min for tube test
- 13 min for DiaClon ABO/Rh for Newborns
- 22 min for DiaClon ABO/D + Reverse Grouping

Summary/Conclusions: The STATUS1 ABO/D typing cards offer a simple, reliable, rapid blood grouping technique which is significantly faster than CAT. It did not detect the Weak D's, as relevant in an emergency situation. It offers a robust, standardised solution to rapid blood grouping for provision of blood in emergency situations with electronic result interpretation and transmission to LIMS.

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THE MDMULTICARD FOR TYPING FY^A, FY^B, JK^A, JK^B, S, AND LITTLE S WAS SUPERIOR TO THE TUBE METHOD

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Background: Phenotyping of red cells normally is done by a haemagglutination test. For many antigens antisera containing monoclonal IgM are available, which allow direct agglutination of antigen positive cells. For several antigens only IgG antisera exist, which require an indirect antiglobulin technique. Recently, the MDmulticard Basic Extended Phenotype for typing red cells for Fy^a, Fy^b, Jk^a, Jk^b, S, or s antigens was developed, combining IgM and IgG antisera in one technique.
Aims: In this study we tested the card for its reliability and for practicability in laboratory routine.
Methods: Blood samples from 45 donors and 40 patients were investigated. The patients had not been transfused 3 months prior to this study. The patient samples included 10 samples with a reactive direct antiglobulin test (DAT) and 5 cord bloods. All patient samples were phenotyped by the tube method for Fy^a, Fy^b, Jk^a, Jk^b, S, and s according to the manufacturers' instructions. All samples with a reactive DAT and all samples with discrepant results were genotyped for *FY*A, FY*B, FY*X, JK*A, JK*B, S* and *s* using commercially available SSP-PCR Kits. The donors had been phenotyped or/and genotyped at earlier occasions. All samples were phenotyped by the multicard. The blood was washed once with saline, then 1 drop of sediment blood was mixed with 8 drops of Diluent F. Two drops of the resulting suspension were added to the application zone of the multicard. After 30 seconds, 6 drops of Diluent F were added to the application zone. After 4 min, another 6 drops of Diluent F were added to the application zone. The results were read and recorded after 4 min (8.5 min after application of the suspension to the card). The results of both methods were compared.
Results: The test procedure was easy to perform and the results were available after a few minutes. 510 antigens were typed, 6 discrepant results were observed: When phenotyping patients with a reactive DAT, two samples were typed Fy(a+) by the tube method and Fy(a-) by the multicard. Genotyping showed that both lacked the *FY*A* allele and confirmed the results of the multicard. Two patients, one newborn, and one donor were typed Fy(b-) by the tube method, but Fy(b+) by the multicard. Genotyping of the four samples revealed the *FY*X* allele which causes a weak Fy^b phenotype. The multicard results were therefore correct.
Summary/Conclusions: The handling of the MDmulticard was suitable for our routine laboratory. Its performance in this study was superior to the standard tube method in two ways: For Fy^a only IgG antisera for the indirect antiglobulin test are available. Therefore two samples with a reactive DAT gave false positive results. With the multicard, containing IgG antisera as well, these samples were correctly typed as negative. Weak Fy^b phenotypes were overlooked by the tube method, but were easily detected by the multicard. Thus the multicard may be a reliable and rapid method for extended phenotyping, e.g. for patients with warm reactive autoantibodies and for patients with sickle cell disease.

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THE IMPORTANCE OF ADSORPTION TECHNIQUE FOR AIHA PATIENTS (ALLO-ANTIBODIES MASKED BY AUTO-ANTIBODIES)

S Gihad

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Background: Warm auto-antibodies are directed against patients' own red blood cell antigens and can interfere with and complicate investigations for the detection and identification of RBC allo-antibodies. Most patients with AIHA have already been transfused and the patients' phenotype can be difficult to determine.
In warm type AIHA; the auto- antibodies in the patient's serum react with all normal red blood cells and make it impossible to find compatible blood.
Special appropriate compatibility test procedures in a reference laboratory allow the detection and identification of clinically significant allo- Abs that may be masked by the auto- Abs.
Aims: The aim of the study is to clarify the importance of implementation of adsorption techniques as a routine pre-transfusion testing in Egyptian patients with warm type AIHA, for detection of co-existing allo-Abs which are present in a high percentage of our patients for safety and effectiveness of their blood transfusion.
Methods: The study done on 300 patients with warm type AIHA, presented to the Red Cell Reference Lab at Egyptian NBTS.
According to the Egyptian national testing strategy; routine lab investigations in the form of screening for red cell Abs and auto-control were done for all these patients and revealed positive auto-control with pan-positive reactivity with different phenotyped RBCs, using Column Agglutination Technique.
According to our testing algorithm; these patients subjected to allo-adsorption; in this procedure adsorption of auto-antibodies from the patient's serum is carried out using three to four samples of allogeneic red cells of varying phenotypes, these phenotyped samples already prepared monthly from selected blood units for this purpose. These samples should be complementary lacking the clinical significant red cell Ags, and are used to adsorb auto-antibodies from the patient's serum at 37°C on several phases to ensure complete successful adsorption of auto-antibodies. The adsorbed serum then tested for allo-antibodies.
Although auto-adsorption technique is much easier than allogenic adsorption in interpretation, it was not achieved due to deficient enough patients' samples and also most of the patients were recently transfused making auto- adsorption inappropriate.
Results: Of the 300 patients; 69 (23%) revealed co-existing allo-antibodies. Of the 300 patients, 231 (77%) revealed only auto-Antibodies without no co-existing allo-antibodies.
Clear identification of the masked coexisting allo-Abs directed us to transfuse the patients with Ag/s free blood units for the relevant identified Ab/s, and hence we avoided the risk of hemolytic transfusion adverse effect of these allo-Abs.
This changed the previous wrong concept of transfusion of the least incompatible blood units to patients with AIHA.
Summary/Conclusions: Proper management of patients with AIHA requires the implementation of techniques that can differentiate between auto- & allo-antibodies and allow the identification of co-existing allo-antibodies which are present in a high percentage of patients with AIHA.

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UK NEQAS (BTLP): EQA AS AN OPPORTUNITY FOR EDUCATION

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Background: The scheme provides EQA to 339 laboratories in the UK, and another 372 laboratories worldwide, for ABO/D, crossmatching, red cell phenotyping, and antibody screening/identification, with interpretations assessed and scored. Additional information is collected regarding reaction grades and processes, and 'non-scoring' elements, e.g. samples with a dual population for ABO and/or D, and 'emergency' scenario exercises are included. This allows trends to be identified and educational points, both general and specifically relevant to UK practice, to be highlighted. In October 2016, an EQA plasma pool containing anti-C was found to be contaminated with weak anti-D (not detectable by Indirect Antiglobulin Testing (IAT)). This was issued as a deliberately non-scoring sample as the anti-D element did not meet specifications for scoring, and no associated clinical details were provided to participants. The aim was to make educational points on interpretation of antibody identification had this been a female with child bearing potential. UK (BSH) guidelines for testing in pregnancy make recommendations for further testing where apparent anti-D+C is identified, and the need to categorise any anti-D detected as either immune, or prophylactic immunoglobulin.
Aims: To investigate the reporting of a very weak anti-D in an EQA sample, and as part of a wider educational remit, to highlight the risks of making an interpretation of anti-D in a specific patient group.
Methods: An EQA sample containing anti-C (2+ by IAT), and anti-D which was not detectable by IAT was issued to 494 laboratories in 24 countries. Results were analysed based on antibody(ies) specified, and antibodies not excluded.
Results: All 446 laboratories performing identification reported anti-C. 271/446 (60.8%) reported anti-C only, 103/446 (23.1%) anti-D+C, 5/446 (1.1%) that anti-D could not be excluded, and 22/446 (4.9%) stated that another antibody with undetermined specificity. 17/446 (3.8%) laboratories incorrectly identified the second antibody, with 15 reporting anti-E, one anti-C^w, and one anti-Kp^a.
Summary/Conclusions: Reactions with D positive, C negative cells in an apparent anti-D+C could be due to anti-G rather than anti-D, especially where the anti-C titre is greater than anti-D, as in this sample. During pregnancy, it is important to perform further testing to confirm whether anti-D is present, to ensure appropriate access to anti-D prophylaxis, if required. Although not currently stated in UK guidelines, this should include all females with child bearing potential to prevent future pregnancies being compromised. Weak anti-D may be present due to administration of passive anti-D immunoglobulin, and reporting the presence of immune anti-D in the patient record could risk denial of access to anti-D immunoglobulin prophylaxis in the future. It is not uncommon for weak anti-D to react preferentially with R_2R_2 cells, as these have a high D antigen site density, making it difficult to differentiate between weak anti-D and weak anti-E reacting only with the 'double dose' of the E antigen on R_2R_2 cells. The EQA report was used as a platform to highlight some risks when reporting antibody identification results in a pregnancy, as identified in recent UK guidelines, and an educational point was included for those misidentifying anti-D as anti-E.

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ABO AND RH PHENOTYPE FREQUENCIES IN MACEDONIAN BLOOD DONORS

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Background: In addition to ABO and RhD, Rh (C,c,E,e) and Kell antigen typing is performed in about 15% of the blood donors in order to have pre-typed red blood cells (RBC) and to provide donors with specific phenotype for alloimmunized patients. This strategy has proved to be useful and cost-beneficial because compatible RBC units are provided on time for most of the alloimmunized patients and with less laboratory testing. Large scale RBC typing beyond the routine ABO/RhD is also a basic prerequisite for the establishment of national rare blood group register.
Aims: The aim of this study is to compare the currently estimated RBC antigen frequencies with the one estimated in 2009 and for the first time to assess the frequencies of Rh phenotypes in our blood donors.
Methods: Statistical analysis was performed on the results of the ABO, Rh, and Kell testing (microplate automated system from Bio-Rad) of 23,501 blood donor samples. Data were obtained from the information system which was established in April, 2016.
Results: The current frequency of ABO blood groups vs the one estimated in 2009 is the following: 41.1% vs 39.7% for A, 33.1% vs 38.5% for O, 17.3% vs 14.2 for B and 8.3% vs 7.5% for AB. Rh phenotype and Kell typing was performed on 3,085 (13.1%) of blood donor samples and the current frequency of Rh antigens vs the one estimated in 2009 is the following: 86.0% vs 79.2% for D, 75.2% vs 58.3% for C, 27.0% vs 21.3% for E, 75.0% vs 82.4% for c and 97.6% vs 97.1% for e antigen. The frequency of Kell antigen is 7.1% vs 6.3% in 2009 with no case of homozygous expression. The most frequent Rh phenotype is DCcee (34.2%), followed by DCCee (24.8%), DCcEe (14.8%), dccee (11.5%), DccEe (9.5%), DccEE (2.3%), dccEe (0.2%), DCCEe (0.06%), dCCee (0.03%) and DCcEE (0.03%).
Summary/Conclusions: The observed differences in the current and the previously estimated Rh antigen frequencies is probably due to the greater demand of RhD negative blood and consequently the greater number of RhD negative blood donor units tested. Further testing on larger number of blood donors should be performed to target the possible demographic changes in blood donor population which may also play role in the observed ABO and Rh antigen frequency differences. This is particularly important for the establishment of rare blood group registry from which people with rare phenotypes and patients with multiple RBC alloantibodies would benefit most.

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SCREENING DONOR BLOOD BAGS FOR POTENT RBC ANTIGENS FOR DECREASING THE RISK OF ALLOIMMUNIZATION IN MULTI TRANSFUSE PATIENTS

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Background: Alloimmunization is one of the side effects of regular blood transfusion in thalassemic patients. These alloantibodies against the minor blood groups which two-third of them are against Rh and Kell subgroups. We studied the frequency of Kell antigen in donor blood bags used in Adult Thalassemia Clinic in Tehran. Our Blood units is comes directly from Iranian Blood Transfusion Center,So such frequency assessment can shows the frequency of Kell Antigen in blood donors.
Aims: Our aim was screening of Kell antigen on the blood bags who came for use in thalassemia clinic.
Methods: First, we record the bag number and with this number we could access to the characteristic of the blood donor, such as sex, age, number of donation,and so on. Then for Kell study, we used anti Kell kit on one of the cords of the blood bag. The results collected and analyzed by SPSS18.
Results: In our one year cross sectional study, we checked Kell Antigen for 11,557 blood bags. 98.7% male, 1.3% were female. Minimum age of our donors was 17 yr and maximum 65 years old. 19.6% were first donor, 27.4% had history of donation before and 52.9% were repeated donors. In our kell study for K or KEL1 antigen we had 96.2% Kell negative and 3.8% of our blood bags were Kell positive.
Summary/Conclusions: We had the rate of less than 4% positivity of Kell antigen on our donors,So more than 96% were Kell neg So for the reduction of the risk of alloimmunization in chronic transfusion patients, it is better to Screening all bags for Kell antigen and do not use Kell Positive bags for such high risk patients.

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DETERMINATION OF RHD VARIANTS AT THE BLOOD TRANSFUSION CENTRE OF SLOVENIA BETWEEN 2013 AND 2015

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Background: RhD (D) antigen is the most immunogenic and clinically important in the Rh blood group system. Serologically, D can be D+, D– and D-variant (D-var). D variants include weak D and partial D, all together more than 200 known D-var. In order to prevent D alloimmunization, serological determination of D-var with commercial assay was introduced in 2008 at Blood transfusion centre of Slovenia (BTCS); in selected cases, it was supported by *RHD (D)* genotyping. Pregnant women serologically determined as weak D are treated as D+ and those with partial D as D-. D-var patients are transfused with D– red cells.
Aims: Before the implementation of routine *D* genotyping for all D-var patients and pregnant women, we retrospectively re-evaluated all serological results of D-var typing from the period between 2013 and 2015 and compared them with genotyping results, where both methods were used.
Methods: A total of 416 samples were serologically tested for D-var using a commercial assay ID-Partial RhD Typing Set (BIO-RAD). 99 cases were additionally genotyped with a PCR-SSP method RBC-Ready Gene CDE, RBC-Ready Gene D weak and RBC-Ready Gene D AddOn (all Inno-Train).
Results: 201 pregnant women (48.3%), 65 patients (15.6%), 40 newborns (9.6%) and 110 donors (26.5%) were included in the study. The serological results were as follows: 252 weak D (60.6%), 66 inconclusive (15.9%), 40 D+ (9.6%), 29 partial D (7.0%), 17 D-var (4.1%) and 12 D– (2.9%). 99 out of 416 (23.8%) were genotyped in order to confirm or to clarify indeterminable serological results (63 and 36 cases respectively). The testing of 38 serologically weak D samples confirmed the presence of *weak D type 1* in 22 samples (57.9%), *weak D type 2* in 3 samples (7.9%), *weak D type 3* in 10 samples (26.2%) and *weak D type 15* in one (2.6%) sample. In one sample, *weak D* could not be determined and another sample showed an unusual genotyping pattern result. The genotyping of 21 serologically typed partial D samples confirmed category *DVII* in 13 (61.9%), *DVI* in 6 (28.6%) and *DIV.04* in 2 (9.5%) cases. 13 out of 36 (36.1%) serologically inconclusive cases were genotyped as *weak D type 3*, 3 as *weak D type 1* (8.3%), 2 without *D* gene (5.6%) and one sample as *weak partial D 4*, *DIV.04, DV* and *DFR* (2.8%). In one sample, we were not able to determine the *D* status. The remaining 12 samples showed the presence of the *D* gene, but weak *D* type could not be determined.
Summary/Conclusions: Our analysis showed that 8% of serologically determined weak D are not *weak D types 1, 2* and *3* and should as pregnant women be treated as D– according to the data from the literature. Routine molecular typing of all D-var pregnant women and patients will provide more accurate information and consequently increase safety of pregnant women and enable preserving D– stock in patients with *weak D types 1, 2* and *3*.

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Abstract has been withdrawn.

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Abstract has been withdrawn.

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INCOMPATIBLE CROSS MATCH – FIRST SIGN OF A HAEMOLYTIC TRANSFUSION REACTION DUE TO INCOMPATIBLE PLATELET TRANSFUSION

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Background: Our hospital is a hemo-oncology center in eastern India and we have many chemotherapy patients and transplant patients who are transfusion dependent. We report here a hemolytic transfusion reaction due to out-of-group SDP (single donor platelet) transfusion, detected in the laboratory due to an incompatible cross match.
Aims: To determine the cause of an incompatible cross match in a multi-transfused patient. A 56 years lady, a known case of treated carcinoma ovary stage III C, now developed low platelet count and anemia. She was diagnosed as therapy induced MDS for which she received decitabine and was on transfusion support. Patient had received several RBC transfusions (always A positive) and many platelet concentrates, both RDP (random donor platelets) and SDP. Platelet transfusions were not always group specific as it depended on the platelet inventory and availability of SDP donors. We now received request for 2 RBC for this patient.
Methods: Blood group, antibody screen and cross match was done by CAT (Ortho Biovue Microbead System). The AHG cross match was performed using the anti-IgG-C3d polyspecific card. Antibody screening was done using three cell panel (surgiscreen cells) and when positive, further identification was done using 11 cell panel (Resolve panel A). Direct antiglobulin test was done by both tube and CAT techniques. Additional tests – acid elution (BAG – elution kit, Ab Acid elution, Bag healthcare Amtsgerichtsstrabe 1–5, Germany) and antibody titration (master dilution method) were done. Standard validated techniques were used. For isoagglutinin titration, the saline method was used for IgM and AHG method for IgG. The end point for the titration was the highest dilution which gave 1+ agglutination.
Results: Patient blood group was A positive. Cross match with four A positive RBC units were all incompatible. The antibody screen with 3 cell and 11 cell panel was negative and cross match with O positive RBC units was compatible. The DCT and auto control were both positive. Since she was a muti-transfused patient, the transfusion details were sought. In the last 48 h, she had received 4 RDP units (two A

group and two O group) and 2 SDP units (both O positive). Both the SDP donors were called and anti A isoagglutinin titers determined. The anti A titers were 64 saline, 128 AHG in one donor and 128 saline, 256 AHG in the second donor. Retrospectively it was noted that following the SDP transfusions, the haemoglobin dropped from 8.3 g/dl to 7.5 g/dl and unconjugated bilirubin increased from 0.8 mg/dl to 2.2 mg/dl.
Summary/Conclusions: At our center in routine practice, group specific SDP donor is preferred; but when not available, out of group donor is taken. Henceforth, for out-of-group SDP procedures, O group donor is taken only if the antibody titers are less than 64. This report also highlights the fact that out of group platelet transfusions could be the cause of an incompatible cross match in multi-transfused patients.

Red cell immunology: Molecular

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NON ABO RED BLOOD GROUP GENOTYPING IN THALASSAEMIA PATIENTS AT CIPTO MANGUNKUSUMO HOSPITAL (RSCM) JAKARTA

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Background: Red blood cell (RBC) alloimmunization is a significant problem in chronically transfused Thalassemia patients in Indonesia. Antigenic differences between donors and patients are responsible for post-transfusion complications. In transfusion medicine, much time and effort are expended in detecting and identifying blood group antibodies. Next to ABO, the most clinically significant antibodies are those in the Rh, Kell, Duffy, and Kidd blood group systems. Extended blood group genotyping beyond the ABO phenotype may allow not only improved survival of transfused units of RBC but also reduce the need for blood transfusion, the iron overload and address the risk of alloimmunization in Thalassemia patients.
Aims: This study aims to look presentation of antigen on surface red blood cells. So that in patients with multiple transfusions such as thalassaemia receive a blood transfusion in accordance with the red blood cell surface antigens, thereby reducing the risk of transfusion and can improve the quality of life thalassaemia patients.
Methods: Eighty six blood samples from Thalassaemia patients receiving repeated blood transfusions were screened by Indirect Coomb's Test (ICT) and followed by antibody identification. Samples with identified antibodies were confirmed by genotyping technique using ID Core XT (Progenika-Grifols, Derio, Spain). The ID CORE XT is a qualitative kit that covers the following 10 Blood Group Systems: Rh, Kell, Kidd, Duffy, MNS, Diego, Dombrock, Colton, Cartwright and Lutheran comprising of 37 RBC antigens. ID CORE XT technique was then applied to obtain RBC antigen profile of Thalassaemia patients.
Results: Genotype of 10 blood group systems were obtained in 86 DNA patients being genotyped. In Rh blood group system, the majority of the patients (61%) were *RHCE*Ce/Ce*, *RHCE*Ce/RHCE*cE(10)* genotype (19%), *RHCE*ce/RHCE*Ce* genotype (10%), *RHCE*Ce/RHCE*CE* genotype (8%) and only 2% expressed rare *RHCE*ce/ce* and *RHCE*cE/cE*. In Kidd blood group system, the most common genotypes (42%) were *JK*A/B*, *JK*B/B* (17%), *JK*B/JK*B_null(IVS5-1a)* (5%), *JK*A/A* (35%) and the rarest one (1%) was *JK*A/Bnull(IVS5-1α)*. Duffy blood group system showed the majority of *FY*A/A* genotype (79%), *FY*A/FY*B* (20%) and only 1% for *FY*B/B* genotype. Several antigens were observed in MNS blood group system. *GYB*s/s* genotype was the most common (81%), followed by *GYB*S/s* genotype (17%) and *GYPB*S/Mur(Mia)* genotype being the rarest (2%). Other genotypes within this MNS blood group system were 50% *GYPA*M/N* genotype, 35% *GYPA*M/M* genotype and 15% *GYPA*N/N* genotype. Lutheran blood group system showed 99% *LU*B/B* genotype, not call (1%), whilst in Diego blood group system showed 98% *DI*B/B* genotype and 2% *DI*A/B* genotype. In Dombrock blood groups systems 27% were *DO*A/B* genotype, and 73% were DO*B/B genotype, whilst in Colton, Kell blood group system and Cartwright blood group system, 100% each were *Co*A/A*, *KEL*k_KPB_JSB/k* and *YT*A/A*.
Summary/Conclusions: Red blood group genotyping offered satisfactory result in identifying red blood cells antigens including the rare ones. Application of this technique in clinical transfusion practice would undoubtedly beneficial to the patient to get the right and savest donated blood.

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EVALUATION STUDY OF THE ERY SPOT® WEAK D-TYPE GENOTYPING ASSAY ON THE MR.SPOT® PROCESSOR

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Background: In clinical transfusion the Rhesus D Antigen of the Rhesus blood group system is the second most clinical relevant blood group antigen. In the Caucasian population approximately 1–2% of the Rhesus D variants show a diminished, altered or extremely low expression on the surface of RBC which makes their detection with the basic serological phenotyping hard or in some cases impossible. Among the commercial available applications the adsorption-elution techniques can only identify some of the low expressed Rhesus alleles however with a time consuming and laborious procedures. The need for detecting D variants to prevent alloimmunization in blood transfused patients drives the need for an alternative automated genotyping procedure. The automated ERY SPOT® Weak D-TYPE genotyping assay was developed by BAG Health Care to satisfy the increasing typing needs and to overcome the limitations of the serological methods.
Aims: Aim of the current study was the evaluation of the performances of the sequence specific oligonucleotide (SSO) ERY SPOT® Weak D-TYPE kit. Besides the most prominent clinical relevant Rhesus weak D alleles the kit detects both the Rhesus D positive and the Rhesus D negative alleles by means of the automated MR.SPOT® system.
Methods: To assess the kit performances approximately 1,100 serologically and/or molecularly typed Rhesus D samples were tested. Three external centers participated to the study. The DNA isolation was performed on buffy coat and whole blood samples using either magnetic beads or column based DNA extraction methods. Different thermal cyclers were used for the amplification of the target region designated by ERY SPOT® Weak D-TYPE kit's components. The MR.SPOT® Processor was used to perform the hybridization assay ensuring an automated, traceable standardized workflow. The analysis of the results was performed with the HISTO MATCH software. To confirm discrepancies between pre-typing and SSO results, affected samples were retyped using reference methods (SSP-PCR, SBT).
Results: The ERY SPOT® Weak D-TYPE evaluation study performed using MR.SPOT® system revealed 98.5% concordance with SSP results corroborating the specificity of the test. In total 185 of 186 pre-typed weak D typed samples were confirmed by the SSO assay. Beside the predominant clinical relevant altered D variants (n = 11 weak D, psi), the predictive accuracy of true RhD negative and positive alleles was more than 99%. Furthermore, three serologically RHD negative samples were reclassified as RHD weak by the SSO assay. Comparative analysis with SSP technology confirmed the SSO results proving the sensitivity of the assay. The assay showed similar success rates in the three external centers, regardless of the used thermal cycler models.
Summary/Conclusions: This work presents a new genotyping assay to detect clinically relevant Rhesus variants in serologically RHD weak or in RHD- samples using the ERY SPOT® Weak D-TYPE. In three cases, genotyping overcomes the limitation of serology, since RHD negative samples could be reclassified as RHD weak type 1 with a high impact on the transfusion strategy. The discrepancies between serology and molecular typing showed that serologically RHD negative samples are in some cases not correctly typed. The results of the Rhesus typing showed high concordance with the reference methods. All tested parameters show the expected results indicating that the system is robust and precise. Taking all findings into account, the new ERY SPOT® Weak D-TYPE kit provides a precise, fast and automated tool for high throughput screening and typing of Rhesus alleles.

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MOLECULAR TYPING AND FREQUENCY INVESTIGATION OF 37 BLOOD GROUP ANTIGENS IN KOREAN BLOOD DONORS RECRUITED NATIONWIDE

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Background: Transfusion recipients who have unexpected alloantibodies must receive corresponding specific antigen negative blood to prevent hemolytic transfusion reaction. In comparison to serological method, molecular typing for blood group antigen can be used when there is no available antiserum or the expression of red cell antigen is weak. Blood group genotyping also has some advantages such as no bias among testers and being able to test various types of antigens simultaneously for many samples. Because of these characteristics, genotyping method for blood group antigens has high throughput capability and is suited for mass screening donors. In this study, we conducted red cell antigen typing on Korean blood donors recruited nationwide using a blood group genotyping system and investigate the prevalence of various red blood cell antigens.
Aims: The purpose of this study is to provide information about the frequency of various red blood cell antigens in Korean population and to establish a rare donor registry to supply specific-antigen-negative blood components.
Methods: Blood donors aged 19 to 54 were recruited at the Korean Red Cross blood centers located nationwide with informed consents. Peripheral venous blood was collected from them and genomic DNA was extracted by the MagNa Pure 96 (Roche). Molecular typing was performed by using the RBC genotyping system ID CORE XT (Progenika Biopharma). For each donor, 37 blood groups belonging to 10 blood group systems were identified except ABO and RhD. Then we determined the frequency of each red cell antigens and chose the target blood types with the prevalence of less than 1.0% for the registry of rare blood.
Results: RBC genotyping was performed on 4,407 samples till February 28, 2017. Among 37 blood group antigens tested, 13 antigens were determined as high frequency antigen with the prevalence of 99% or more in Korean population (k, Kp^b, Js^b, Fy^a, s, U, Di^b, Do^b, Hy, Jo^a, Co^a, Yt^a, and Lu^b). Some blood type combinations such as Jk(a-b-), Fy(a-b-), and S-s- were not found in any individual and were included in the rare blood criteria.
Summary/Conclusions: The rare blood types which accounts for less than 1% of Korean population are similar to those of neighboring countries. In particular, the frequencies of K, Jk(a-b-), and S-s- are different from the previous reports and are rather similar to those in Chinese or Japanese. The significance of this study is additional accumulation of data on the frequency of blood group antigens through highly accurate molecular typing in the East Asia region. We identified rare blood types of RBC antigens in Koreans, which account for a decent percentage of the population in this region. And we established the registry of rare blood donors.

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STANDARDIZATION OF AUTOMATIC PROTOCOLS FOR IDENTIFICATION OF RARE BLOOD DONORS COMPATIBLE FOR IMMUNIZED PATIENTS

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Background: In most cases patients immunized to high prevalent erythrocyte antigens may only be transfused with red blood cells from rare donors with no such antigens. The identification of such donors with serological tests is rather difficult due to the lack of commercially available reagents and low throughput testing. High throughput molecular methods used for genotyping of blood groups permit identification of donors with rare blood group and justify establishment of rare blood donor registry for both transfusion and diagnostic purposes. The number of rare blood donors entered into the Polish registry is still rather low and it is necessary to rely on low cost (*home-made*) methods whenever necessary.
Aims: Standardization of protocols for genotyping $Yt^{a/b}$, $Kp^{a/b}$, $Do^{a/b}$, $Di^{a/b}$, $Co^{a/b}$, $LW^{a/b}$, FY GATAbox (+)/(-), $Wr^{a/b}$, $Lu^{a/b}$, $Kn^{a/b}$ i Vel (+)/(-) polymorphisms encoding rare antigens.
Methods: DNA from whole blood of 808 blood donors from Regional Blood Transfusion Centres was isolated automatically using Chemagic DNA Blood Kit LH (Chemagen) with Janus pipettor (Perkin-Elmer). Allelic discrimination of 11 polymorphisms was performed automatically by real-time PCR (LightCycler 480, Roche).
Results: 11 real-time PCR protocols were developed for identification of polymorphisms encoding rare antigens, which can be used in fully automated procedure on microplates in 96-well format. Among 223 blood donors used for validation of automatic protocols two rare donors with *YT*B/B* genotype (confirmed by serological testing) were identified. In the remaining samples rare genotype was found only in heterozygous configuration. The estimated frequency of tested rare alleles in Polish population is as follows: *YT*A* 0.917/*YT*B* 0.083; *KP*A* 0.011/*KP*B* 0.989; *DO*A* 0.64/*DO*B* 0.36; *DI*A* 0.003/*DI*B* 0.997; *CO*A* 0.93/*CO*B* 0.07; *LW*A* 0.981/*LW*B* 0.019; *FY GATAbox (+)* 1.0; *VEL*(+) 0.98/*VEL(-)* 0.02.
Summary/Conclusions: The developed protocols enable fully automatic 96-format screening of 11 rare blood group alleles and will be used for identification of rare blood donors.

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Abstract has been withdrawn.

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MALDI-TOF MS ANALYSIS OF 36 BLOOD GROUP ALLELES AMONG 398 THAI SAMPLES REVEALS REGION-SPECIFIC VARIANTS

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Background: Blood group phenotype variation in different populations has been attributed to potential pathogen resistance. We wanted to investigate the blood group antigen distribution in two groups of blood donors from different regions of Thailand, one north (Lampang) and one central (Saraburi), to map variation.
Aims: To characterise the blood group allele profile of Thai blood donors by MALDI-TOF Mass Spectrometry (MS) and correlate with phenotype.
Methods: Genomic DNA from 398 Thai blood donors was analysed by a MALDI-TOF MS platform that targets 36 blood group related SNPs in 15 systems. The results were compared with serology, and all discrepancies were investigated in closer detail. An allelic discrimination (AD) assay was used to analyse a regulatory SNP (rs1175550) in *SMIM1*.
Results: Serological results were available on all samples for Rh, MNS, and K. Genotyping/phenotyping for K, and S/s showed 100% concordance. Genotyping predicted the correct RhD and RhCE phenotypes in 100% and 99.2% of all samples tested, respectively. Serological investigation of 3 outliers with a panel of monoclonal anti-e revealed an e-variant antigen. Sequence analysis identified heterozygosity for the *RHCE*02.22* allele in these samples. This allele had been shown previously in Caucasians associated with a weak C antigen expression.
Discrepancies with MN typing in 44 samples revealed glycophorin variants of which 41/44 were Mi(a+). Three Mi(a-) outliers were sequenced at the junction of *GYPB* exon 3/intron 3. No apparent hybrid was identified in these samples. Analysis of cDNA is on-going. Nine samples (2.3%) carried the weakening mutation, c.265C>T on an *FY*01* allele, of which six were identified in blood donors from Lampang. All nine samples were homozygous for *FY*01*, thus weakened expression of Fy^a antigen was not observed. Six samples (1.5%) were heterozygous for the *JK*02N.01* allele, of which five genotyped as *JK*02/JK*02N.01* and one as *JK*01/JK*02N.01*. The latter typed Jk(a+b-). The null alleles were more common in the central region (5/6 samples).
Other findings revealed only 2/398 K+k+ samples. Interestingly, MALDI-TOF MS revealed 5 samples to be *DI*01/DI*02* which was confirmed serologically. Of these, 4 were blood donors from Saraburi. One blood donor genotyped as *IN*01/IN*02*.
All samples were homozygous for wild type *SMIM1*. AD of rs1175550 revealed homozygosity for the AA allele in 216/223 samples tested (97%), the remaining 7 samples genotyped as AG.

Summary/Conclusions: MALDI-TOF MS is an efficient method for rapid genotyping and correlated well with the phenotype. We observed the expected high prevalence of the Mi(a+) phenotype in donors from both regions. However, other variants were identified indicating further diversity at this locus. Interestingly, nine samples (2.3%) carried the c.265C>T mutation on a *FY*01* allele. This allele is commonly associated with *FY*02* in other parts of the world but was recently reported in the Thai population. The Duffy protein is a known ligand for *Plasmodium vivax* which is endemic in this region. The prevalence of *FY*01* is 98.44% in our cohort and one may speculate that modification of this protein by c.265C>T may reduce susceptibility to invasion. This remains to be investigated.

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COMPARISON OF BLOOD GROUP GENOTYPING TO SEROLOGICALLY DETERMINED PHENOTYPE AND PREVALENCE OF ALLOIMMUNIZATION IN CHRONICALLY TRANSFUSED THALASSEMIC PATIENTS

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Background: Chronically transfused patients are at risk for alloimmunization, thus it is important to define their erythrocyte antigenic profile in order to select matching blood to transfuse them. Determining blood group antigens by serological methods could be unreliable in multi-transfused patients. Red blood cell genotyping is considered a safer approach for phenotype determination in such patients.
Aims: To evaluate the utility of blood group genotyping in routine transfusion practice and the effect of its implementation in prevention of alloantibody formation in chronically transfused thalassemic patients.
Methods: Genomic DNA from 35 thalassemic patients was isolated from the buffy coat and ABO genotyping was carried out by Sequence Specific Primers (SSP)-PCR kits (RBC-Ready Gene ABO, CDE, KKD, inno-train Diagnostik GmbH). In all of them RhD, RhCE and Kell phenotype and in 17 Kidd phenotype had previously been determined serologically with hemagglutination (CE-Immunodiagnostica GmbH). The results of both methods were compared. Prevalence of alloimmunization was recorded.
Results: In 14 patients (40%) the serological and the molecular-predicted Rhesus phenotypes were discordant. In 58% (10 out of 17) of the samples tested for Kidd, molecular and serological results were discordant.
Overall 17 alloantibodies were detected in 10 patients (28%). Most of the antibodies were against Kell system (anti-Kell, anti-Kpa). Rh antibodies (anti-D, anti-C) were found only in one Rh-negative patient. One antibody was identified as antibody against rare antigen and one remained unidentified.
In spite of the fact that 42% of the discordant Rhesus phenotypes had the potential for alloimmunization, no antibodies against Rhesus antigens were found.
No antibodies against Kidd antigens had been evolved (or detected) although 40% of the discrepancies had the potential to evolve antibodies and two patients had been systematically transfused with the "fault" antigenic blood before genotyping was implemented.
Five out of 18 antibodies (28%) had been previously apparent (from patient's records) but were not currently detectable.
Summary/Conclusions: Despite large scale discrepancies between molecular and serological Rh phenotype determination in our patients and the fact that they had been transfused with the 'fault' antigenic blood for many years, the Rh-alloimmunization prevalence in our patients was low, and the Kidd alloimmunization incidence was null. This implies that other factors, apart from antigenic exposure, contribute to immunoreactivity. Transfusion with serologically determined phenotype for years did not adversely affect our thalassemic patients in respect to antibody formation. These results query the necessity of red blood cell genotyping in routine transfusion practice in already heavily transfused patients although it is of clinical value if done before the first episode of transfusion and it is very helpful in the identification of suspected antibodies.

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DI*A AND DI*B ALLELE FREQUENCIES IN THAI BLOOD DONORS

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Background: Diego (DI) blood group genotyping is important in transfusion medicine as anti-Di^a and anti-Di^b can be associated with delayed hemolytic transfusion reactions and hemolytic disease of the fetus and newborn, especially in Asian populations with Mongolian ancestry.
Aims: This study aimed to determine *DI*A* and *DI*B* allele frequencies among Thai blood donors and to compare them with other populations previously reported.
Methods: Altogether, 1,888 DNA samples obtained from 427 southern Thai, 1,161 central Thai and 300 northern Thai blood donors were genotyped for *DI*A* and *DI*B* alleles by polymerase chain reaction with sequence-specific primer (PCR-SSP). In addition, the allele frequencies were compared with other populations.
Results: It was found that *DI*A* and *DI*B* allele frequencies were 0.0047 and 0.9953 in southern Thais; 0.0181 and 0.9819 in central Thais and 0.0183 and 0.9817 in northern Thais, respectively. The *DI*A* and *DI*B* allele frequencies in southern Thais were similar to those in Filipino, Italian, American native, Hawaiian/Pacific Islander and Alaska Native/Aleut populations; while, the frequencies significantly differed from central and northern Thais ($P < 0.05$).
Summary/Conclusions: This is the first study to report *DI*A* and *DI*B* allele frequencies in Thai populations, which is beneficial for typing multitransfused patients, antigen matching patients and providing antigen-negative RBC donor units to prevent both alloimmunization and adverse transfusion reactions.

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INTEGRATING RED BLOOD CELL GENOTYPING INTO THE REFERENCE LABORATORY: BENEFITS TO CHRONICALLY TRANSFUSED PATIENTS

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Background: Chronically transfused patients with multiple antibodies and warm autoantibodies present complex challenges for hospital transfusion services and reference laboratories. Advances in red blood cell genotyping have increased the options available to reference laboratories for resolving incomplete or inconclusive results for serological testing reducing delays in patient care associated with time-consuming serological workups.
Aims: The aim of this study is to share the experience gained from molecular typing of chronically transfused patients in a immunohematology reference laboratory.
Methods: Over a period of 6 months, we performed molecular analysis in 34 DNA samples from chronically transfused patients with invalid or discrepant results in serologic tests. Genomic DNA was prepared and genotyping was performed using laboratory developed tests (LDTs), HEA BeadChip, RHD BeadChip, RHCE BeadChip (Bioarray, Immucor) and sequencing.
Results: Of the cohort, 22 patients required extended genotyping, 7 required RHD and RHCE genotyping and 5 required RHD sequencing. Of the 22 extended genotyping performed, 9 patients had been recently transfused, 4 patients had multiple myeloma and were receiving anti-CD38 therapy, 4 patients had warm autoantibodies and 5 patients had antibodies to high frequency antigens. Of the 7 RH genotyping performed, 2 patients were phenotyped as RhD-positive with anti-D and 5 patients typed as e-positive with anti-e. In five patients with weak D expression or discrepancy in RhD typing, direct sequencing was performed and 2 novel *RHD* alleles were identified. All cases were successfully resolved by the integration of serological and molecular methods. Patients were transfused with compatible donor units based on the genotype results and benefited of the received transfusions as showed by a good *in vivo* survival. Additionally, genotyping also shortened subsequent antibody workups by an average of 51% and decreased turnaround time for RBC transfusion.
Summary/Conclusions: This study provides evidence that the information gained from molecular typing of chronically transfused patients improve the ability to find highly antigen-matched RBC components for transfusion support decreasing the risk

of delayed hemolytic transfusion reactions and preventing alloimmunization. Besides, RBC genotyping improves customer service by reducing the number of costly serological workups, including adsorptions, and allows faster turnaround time for transfusion.

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ADVANTAGE OF MOLECULAR TYPING OVER SEROLOGICAL METHODS IN SOLVING DISCREPANT RESULTS OF WEAK AND PARTIAL D IN UPPER EGYPT

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Background: About 18% of the European population is D negative, usually because of deletion of the RHD gene and occasionally because of mutations in the RH locus. Two previous studies were performed in Egyptian population to detect the different types of weak and partial D. More than 50 different Rh antigens have been identified by investigating the specificity of antibodies produced after blood transfusion or pregnancy.

Aims: In our study we investigated different types of weak and partial D using both serological and molecular methods tried to solve the vague obtained results by serology. In addition, improve transfusion services related to weak D.

Methods: Nonconclusive results of D phenotyping were adjusted to routine serology in 130 blood donor and patient samples to define the D phenotype including monoclonal anti-D immunoglobulin M and indirect antiglobulin test. Panels of monoclonal anti-D (Diamed, commercially available kit) were used for identification of partial D and weak D phenotypes. DNA was estimated using allele-specific amplification polymerase chain reaction with sequence specific primers to define weak D type.

Results: Molecular typing confirmed vague serological results; we can't define 15 samples using serological methods. All samples were recognized by molecular typing, eight samples were weak D Type 4.0. Four samples were recorded as weak D Type 4.2 (DAR). Lastly 3 samples identified as partial D Type II by serology known as D Type 4.0 by molecular typing. The highest detected percentage was weak D Type 4 in our locality.

Summary/Conclusions: Molecular detection of different types of weak and partial D is superior over serological method. All discrepant weak D results obtained by serological detection were solved by use of molecular technology. Analysis of weak D by molecular types of Rh antigens will decrease consumption of low frequent negative RH blood, improve our prevalence data and increase our knowledge about known alleles.

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MOLECULAR BIOLOGY AS A SUPPORT IN THE IDENTIFICATION OF DONORS/PATIENTS RHD NEGATIVE: EXPERIENCE OF TRANSFUSIONAL SERVICE OF ASL CASERTA

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Background: The molecular biology techniques provide the opportunity to clarify the relationship between erythrocyte phenotype, and their genotype. (Molaro, Reali, Blood Transf, 2003). Particularly in the Rh system, often the phenotype serologically determined does not match the results of molecular investigations. About 18% of the European population is RHD negative for the absence of the RHD gene while 1% is characterized by abnormal RHD alleles that determine a different antigenic reactivity not identifiable with only serologic methods. A particular phenotype is given by the D "partial" (or D "mosaic" or D variant), the D "weak" (weak D, once called Du) or D-DEL.

Aims: The aim of this study was to type by serological methods and molecular biology all donors and patients RhD negative of the Transfusion Service of ASL Caserta in the 2015–2016. In this population, the prevalence of certain antigenic variants was assessed and a immunohematological validation path for categories was defined.

Methods: In the Transfusional Service, gel microcolumn testing (Autovue Innova Ortho Clinical Diagnostics) and solid-phase red cell adherence assay (Galileo, Immucor) were performed to type blood donors, while patients are typed exclusively by gel microcolumn testing (IH-1000 Bio-Rad). All subjects RhD negative or with possible variations of RHD were successively analyzed with RHD BeadChip IMMUCOR.

Results: In 2015 we have 33,637 donors/patient and in 2016 35,870. 15 ± 2% of the donors/patients result RHD negative, while 0.73% result with RHD variants. The RHD variants consist in: D-weak type 1 or 2 or 3 (86%), D-DEL (1%) and undetectable variants (impossibility to identify the RHD variation with the kit in use but the gene is presence in its not intact structure, (13%).

Summary/Conclusions: The molecular biology techniques have definitely improved the genotyping of RH negative donors, because serologic methods are not able to identify all variants. Medical reports, biological validations and best transfusion practices are the steps to be taken more into account. It's imperative to identify donors who have a low antigenicity D and consider them RHD positive: their blood should not be transfused to patients RhD negative because it's not known the immunizing density (Perrone, Blood transfusion 2002). As for the receivers and pregnant women, it would seem more appropriate to classify RHD negative if they do not react directly with the antiserum anti-D, to avoid immunizing events. To predispose a suitable transfusion strategy it would be appropriate in our view to take into account the frequency in the population of weak D and qualitative changes in their antigen, in conjunction with the recording of immunizing events observed. To ensure greater safety in the determination of the Rh factor is definitely necessary to integrate more methods, The molecular biology techniques it is currently the most effective method to validate the result obtained.

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CONTRIBUTION OF ERYTHROCYTE GENOTYPING TO IMMUNOHEMATOLOGY AT BLOOD TRANSFUSION CENTER OF ANNABA, ALGERIA

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Background: Typing of red blood cell RBC polymorphisms at the DNA level is important in transfusion medicine to create an inventory of donor units suitable for patients with rare phenotypes and to select appropriate blood units for multitransfused patients. Furthermore, it is useful to identify donors for the preparation of reagents in RBC panels used to detect or identify antibodies.

Aims: The main objective of our study was to perform erythrocyte genotyping in the Blood Transfusion Center of Ibn Roch Hospital of Annaba.

Methods: A total of 27 regular donors (group O) and 35 multitransfused patients, who are alloimmunized or having a positive antiglobulin test making the serological phenotype difficult to perform, were included. The genotype was performed by using sequence specific primer-polymerase chain reaction (SSP-PCR) method (*RBC-Ready Gene. Inno-train*) in the RH, Kell, Kidd, Duffy and MNS systems and the results were compared with the phenotype results previously performed by classical hemagglutination method.

Results: The results of the genotype and the extended phenotype were the same in more than 85% of the cases. Among discrepancies between the phenotyping and genotyping detected; an incomplete serological phenotype in the Duffy system was deduced from the genotype using silent or mutated alleles. In recent years, several molecular tests for RBCs typing have been used to complement traditional immune hematological assays for pre-transfusion testing. The use of high-throughput DNA analysis was developed to allow the rapid determination of major and minor blood groups, the evaluation of weak antigen expression and identification of rare phenotypes due to specific polymorphisms. Furthermore, the identification of extended RBC antigens is very important for the choice of the best treatment in transfusion-dependent patients.

Summary/Conclusions: Erythrocyte genotyping in immunohematology makes it possible to overcome the limits of hemagglutination and must be realized only when it brings real benefit to the patient and the management of resources.

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BLOOD GROUP VARIANT CALL ANALYSIS FROM A 100-YEAR-OLD LOCK OF HAIR OF AN INDIGENOUS AUSTRALIAN

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Background: Blood groups (BG) are of clinical significance due to their association with transfusion reactions in patients who have been alloimmunised and have made red cell antibodies. This alloimmunised status frequently complicates the provision of compatible blood units for future transfusion needs. Australian blood donors are principally Caucasians; due to differences in BG antigen profiles between ethnic groups, there is an increased risk of BG mismatch, with complications, for patients of other ethnicities. The BG antigens in Indigenous Australians have not been studied since the 1960's. These early studies discovered differences in BGs antigens in Indigenous Australians compared to Caucasians and other ethnic groups. Unique serological typing reagents were developed but have long since been exhausted. The genomic sequence data from a 100-year-old lock of hair of an Indigenous Australian individual from southern Western Australia has been publically available since 2011 (Rasmussen, Science) but the BG profile of this individual has not yet been reported.
Aims: To provide a BG profile from the variant call data for the Indigenous Australian as part of a larger study.
Methods: Variant call format (vcf) files, generated by Rasmussen *et al.* (Science, 2011), were annotated and filtered using CLC Genomics Workbench v.9.0.1 to extract only variants for BG related genes. Variants were manually compared to those in public databases (Erythrogene and NCBI) to identify BG alleles, novel, rare and common variants. Novel and rare variants were further characterised *in silico.*
Results: The vcf files contained 1,496 variants in 37 BG genes (35 systems) and *GATA1*, but no variants in *CD99* (XG system) or *KLF1*. Of the total number of variants the majority were deep intronic, 17 were synonymous and 30 were missense variants. The 30 missense variants included 15 BG alleles, 6 novel variants (GLOB, I, CH/RG, RHAG and 2 in KN system), 5 rare variants and 4 common variants. The vcf files were generated from whole genome sequence data where the breath of exome coverage was reportedly 78.1%. Some BG gene regions can potentially not be covered and we were therefore unable to say whether the absence of variants in a BG gene region signifies a genotype similar to the reference, the presence of a deletion or a region with no coverage.
Summary/Conclusions: Presuming all BG genes have adequate coverage, the predicted phenotype for this individual would be: A_1, D+E+e+, Lu(a+b+), K-k+, Fy(a+b-), Jk(a+b+), Do(a+), I, In(a–b+), Au(a+b+), Kn(a+), KN:-9, FORS1–. The C/c phenotype cannot be called since Exon 2 for *RHD* and *RHCE**C is identical and therefore p.Ser102Pro, essential for *RHCE**C specificity, would not appear in the vcf. Several novel and rare variants with potential clinical significance were identified for further investigation. The results from this pilot analysis will be compared to additional datasets for Indigenous Australians and other populations. Unique BG profiles will then be linked with full red blood cell phenotype and serological findings and used to redevelop serological reagents necessary to identify antigenic compatible blood for Indigenous Australians.

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CHARACTERIZATION OF IMMORTALIZED CELL LINES FOR SELECTION OF RBC GENOTYPING PANEL CANDIDATES FOR RH VARIANTS

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Background: The Rh blood group system is highly polymorphic with more than 250 *RHD* and 100 *RHCE* alleles described. This complexity leads to difficulties for transfusion recipients of African descent especially patients with sickle cell disease at high risk of alloimmunization from demanding chronic transfusion therapy. Use of molecular genetic technology for blood group typing is increasing among reference laboratories worldwide and, consequently, genomic DNA reference reagents to ensure the quality of the tests are needed. We conducted a collaborative study with laboratories around the world to validate a genomic DNA reference panel for covering 41 alleles from 17 blood group systems. Remarkably, the results received from the collaborators showed the presence of *RH* variant alleles among the samples composing the panel.
Aims: Our goal is to perform *RH* characterization of 53 B-lymphoblastoid cell lines generated in the course of the previous study in order to select samples with Rh variants to be included in a renewable blood group genotyping reference panel for the RH alleles.
Methods: To characterize the *RHD* gene of the panel members, we performed *RHD* zygosity testing to check the presence of hybrid, upstream, and downstream Rhesus box by PCR-RFLP and PCR-SSP. In addition, multiplex PCR for exons 3, 4, 5, 7 and 9 followed by PCR-RFLP reactions was used to identify *RHD* variants already described in the literature. The presence of possible novel *RHD* variant alleles and results confirmation were verified by Sanger sequencing. *RHCE* characterization by sequencing is ongoing.
Results: Among the 53 panel members, 42 (79%) were RhD seropositive, and the relevant *RHD* alleles have been characterized. Among the 11 RhD seronegative (21%), 7 (64%) samples had *RHD* deletion, 2 (18%) were *RHD* pseudogene positive (*RHD*psi/RHD*01N.01)*, and 2 (18%) are under investigation. Of the 42 *RHD* positive, 22 (52%) had standard *RHD* allele (*RHD**01) in homozygous state, 13 (31%) had standard *RHD* allele in a hemizygous state, and 7 (17%) had at least one *RHD* variant allele. For the 7 samples with at least one *RHD* variant allele: (a) 2 samples had *RHD*DAU0* allele in homozygous state; (b) 2 samples had one deleted *RHD* allele (*RHD*01N.01)* along with either *RHD*DAU5* or *RHD*DHMi*; (c) 2 samples had one standard *RHD* allele (*RHD*01)* each, one along with *RHD*DAU0* and the other with *RHD*DIII.a;* (d) 1 sample had heterozygous *RHD* variant (compound) comprised of *RHD*DIVa.2* along with *RHD*DAU5*.
Summary/Conclusions: The data generated by Rh investigation will define the allelic composition of the existing CBER panel cell lines and assist with the selection of samples to be included as members of a dedicated renewable reference panel for Rh genotyping.

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USE OF GENOTYPED TEST CELLS IN ANTIBODY IDENTIFICATION PANELS IN THE PRE-TRANSFUSION DIAGNOSTICS

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Background: The identification of antibodies to red cell antigens is one of the most important and challenging issues in transfusion medicine. There are 352 red cell antigens recognized by the ISBT Working Party on Red Cell Immunogenetics and Blood Group Terminology. Most of them belong to one the 36 blood group systems. Some antigens with still unknown genetic background are part of collections, low incidence (series 700) or high incidence (series 901) antigens. The test cells used in commercial antibody identification panels are usually serologically typed for less than 30 clinically most important antigens. Thus the identification of many antibody specificities remains impossible for a number of laboratories.
Aims: We developed antibody identification panels with test cells serologically typed for 26 antigens and additionally genotyped for 28 alleles. These panels used in our routine should improve the pre-transfusion diagnostics extending the range of detectable antibody specificities.
Methods: The antibody identification of all patients was performed in the indirect antiglobulin test using untreated and papain treated red cells in the gel technique. The test cells in the panels were tested serologically for the following clinically most significant "standard" antigens: RhD, C, c, E, e, C^w, K, k, $Kp^{a/b}$, $Fy^{a/b}$, $Jk^{a/b}$, $Le^{a/b}$, P1, M, N, S, s, Xg^a, $Lu^{a/b}$. Following alleles were additionally genotyped by using inhouse PCR-SSP Methods.
*DO*01/*02* (for $Do^{a/b}$), *LU*18/*19* ($Au^{a/b}$), *YT*01/*02* ($Yt^{a/b}$), *DI*01/*02* ($Di^{a/b}$), *IN*01/*02* ($In^{a/b}$), *KEL*06/*07* ($Js^{a/b}$), *LU*08/*14*, *LW*05/*07* ($LW^{a/b}$), *SC*01/*02*, *KN*01/*02* ($Kn^{a/b}$), *KN*03/*06* ($McC^{a/b}$), *KN*04/*07* (SI^a/Vil), *KN*-05* (Yk^{a-}), *KN*-09* (KCAM-), *RHCE*02.09* (C^X), *VEL*-01* (Vel-). Antibodies identified due to the genotype information of the test cells were confirmed by serology using appropriate reference red cells.
Results: 10,727 blood samples of 5,935 different patients were tested in our reference laboratory from August 15, 2014 till February 20, 2017. 93 different patients with following antibodies could initially be identified exclusively due to the information derived from genotyping of the test cells: 4 anti-Do^a, 1 anti-Do^b, 13 anti-Yt^a, 10 anti-Yt^b, 2 anti-Di^a, 2 anti-Lu8, 3 anti-Lu14, 1 anti-LW^a, 1 anti-LW^b, 1 anti-Sc1, 1 auto-anti-Sc1, 1 anti-Sc2, 17 anti-Kn^a, 6 anti-Kn^b, 15 anti-Yk^a, 4 anti-KCAM, 4 anti-Vel, 3 anti-Au^b, 2 anti-MAR-like, 2 auto-anti-MAR-like.

Summary/Conclusions: The use of genotyped test cells in antibody identification panels extends the range of detectable antibody specificities, accelerates the antibody identification and improves the pre-transfusion diagnostics.

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EVALUATION OF ID RHD XT® GENOTYPING TEST FOR IDENTIFICATION OF MULTIPLE RHD ALLELES AND HPA-1A/B

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Background: The Rh blood group system is highly polymorphic and encoded by two closely related genes, *RHD* and *RHCE*, located on chromosome 1. Antibodies toward RhD antigens can be implicated in transfusion complications and haemolytic disease of the foetus and newborn. The RhD protein can be expressed more weakly than normal, so-called weak D, or have some of its epitopes missing, partial D, and for which serological methods sometimes cannot give conclusive results. By determining the *RHD* genotype of patients and pregnant women with weak D phenotypes, unnecessary injections of Rh immunoglobulin (RhIG) and transfusion of RhD-negative blood could be avoided.
Aims: The purpose of this study was to validate the performance of a new assay, ID RHD XT®, using the same platform as already in place at the Nordic Reference Laboratory for Genetic Blood Group Typing for red cell genotyping with ID CORE XT®, against currently used methods for *RHD* genotyping and to evaluate if the results from the new assay could be used for clinical decisions.
Methods: A total of 78 DNA samples, from patients and pregnant women who previously had been genotyped with accredited methods due to inconclusive or weak RhD phenotype according to established serological techniques, were tested with ID RHD XT®. ID RHD XT® is a qualitative, PCR- and hybridization-based genotyping test for simultaneous identification of several alleles (*RHD*01W.1-*01W.3, RHD*08N.01, RHD*03N.01, RHD*01N.01*) of the *RHD* gene and HPA-1 system using LUMINEX® technology. The software BIDS XT version 1.8, an upgrade of the currently used BIDS XT 1.5, was utilized for data management and analysis of results.
Results: Fifty-two of 55 samples (94.5%) gave conclusive results that could be interpreted directly. These data were in full concordance with previously obtained data with the methods used for clinical genotyping in our reference laboratory. No samples gave unexpected or discrepant results. Samples tested included *RHD*01W.01* (n = 11) *RHD*01W.02* (n = 10) *RHD*01W.03* (n = 1) *RHD*06* (n = 3) and other RHD alleles (n = 30). Three samples gave no results (no call). Two of the samples were rerun but still gave no results. Further testing will be performed to see if the DNA has been degraded. Two runs had technical issues, one due to problem with the probe at the LUMINEX instrument and one due to a contaminated water control, consequently the results from these two runs were discarded. The hands-on time process of each of the steps was short and required no extra equipment than was already in place at the laboratory.
Summary/Conclusions: In summary, 52 of 55 tested samples gave easily interpreted results in concordance with previously obtained genotyping results while the remaining samples gave no result. Integration of *RHD* genotyping for certain patient groups and pregnant women in laboratory practices could improve the accuracy of RhD typing results and be of help in clinical decisions, and therefore both reduce unnecessary administration of RhIG in women with weak D phenotypes and decrease unnecessary transfusion of RhD-negative blood to recipients with weak D phenotype.

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PERFORMANCE EVALUATION STUDY OF ID RHD XT, A GENOTYPING ASSAY FOR THE DETECTION OF HIGH-PREVALENCE RHD NEGATIVE AND WEAK D TYPES

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Background: ID RHD XT (Progenika, Grifols) is a qualitative, PCR/Luminex® xMAP hybridization-based genotyping test for the identification of the following *RHD* gene allelic variants: *RHD**weak D type 1, *RHD**weak D type 2, *RHD**weak D type 3, *RHD* deletion, *RHD**Pseudogene and *RHD**DIIIa-CE(3–7)-D and ITGB3 gene: HPA1a and HPA1b, in genomic DNA extracted from whole blood specimens collected in EDTA. The genotype and predicted phenotype results are reported from the combination of the allelic variants tested. It is well established that weak D 1, 2 and 3 phenotypes are not at risk for forming allo-anti-D, whereas a few weak D and all partial D and negative phenotypes are. Routine serologic D typing does not distinguish among them, consequently RHD genotyping is recommended, especially in patients.
Aims: The objective was to evaluate the performance of ID RHD XT genotyping assay in terms of whole system failure rate, call rate and accuracy for Rh and HPA-1 blood groups.
Methods: A cohort of 1,000 previously serotyped samples for D antigen obtained from three European blood centers (Centro Vasco de Transfusión y Tejidos Humanos [Spain], Sanquin Blood Supply [The Netherlands] & Banco de Sangre y Tejidos de Aragón [Spain]) were analyzed with ID RHD XT at Progenika, Grifols. They were distributed as recommended by the Annex of the Common Technical Specifications 2009/108/CE for a IVD product of list A (≥10% Clinical samples, >2% Neonatal Specimens and ≥2% Weak D donors). Due to the intended use of the product, Weak D serotyped donors were enriched (n = 160, 16%). Commercially serology tests for D antigen predicted phenotype and Bi-Directional-Sequencing (BDS) for weak D type confirmation and HPA-1 predicted phenotype were used for comparison.
Results: No system failure, 100% call rate and no inconclusive results were obtained. Discrepancies were found for D antigen between serology and ID RHD XT predicted phenotype results, but a 100% concordance was obtained when analyzed by BDS, considering ID RHD XT result correct. 100% concordance between ID RHD XT and BDS results for the Weak D type determination in 160 samples was obtained. The following ID RHD XT predicted phenotype results were obtained: D- (n = 361), No amplification variant detected (n = 15), Weak D Type 1 (n = 22), Weak D Type 1 heterozygous (n = 1), Weak D Type 2 (n = 32), Weak D Type 2 heterozygous (n = 1), Weak D Type 3 (n = 34), Weak D Type 3 heterozygous (n = 1), Weak D Types 1, 2 or 3 not detected (n = 533). Regarding HPA-1 blood group, the predicted phenotype results obtained by ID RHD XT were 100% concordant with BDS results for the 163 samples compared: HPA-1a positives (n = 157) and HPA-1a negatives (n = 6), HPA-1b positives (n = 46) and HPA-1b negatives (n = 117).
Summary/Conclusions: 100% specificity and 100% sensitivity for D antigen, HPA-1a and HPA-1b antigens were obtained. In this extensive validation, ID RHD XT genotyping assay performed as a reliable and accurate method for predicting the genotype and phenotype of high prevalence RHD negative and Weak D types. That makes it a useful tool for the implementation of the *RHD* genotyping recommendation on patient blood transfusion and anti-D prophylaxis.

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EVALUATION OF VARIANT RHD-GENOTYPING WITH RBC-FLUOGENE KITS FROM INNO-TRAIN

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Background: We wanted to improve the method for genomic *RHD*-typing at our Department because the existing in-house method had its limitations. Genotyping is a useful tool when serology is inconclusive.
Aims: We have evaluated and verified two commercially available genotyping kits from inno-train (Kronberg im Taunus, Germany); RBC-FluoGene CDE and D weak/variant. These two kits in combination cover the most common *RHD*-variants and *RHCE*.
Methods: RBC-FluoGene applies a modified TaqMan-probe system with sequence specific primers (SSPs). The reading of end-point fluorescence was performed with the FluoVista instrument. The following samples were analyzed: nine commercially available controls, five samples with known *RHD*- and/or *RHCE*-genotype, and 46 samples that were typed to be weak RhD positive with a less than 4+ reaction. Of the 46, the Rh-phenotype was available for 16 samples. DNA was isolated with the MagNAPure LC instrument (Roche, Mannheim, Germany) and the inno-train protocols were followed for the RBC-FluoGene kits.
Results: Samples with known *RHCE*- and/or *RHD*-genotypes were in full compliance with the results from inno-train. Among the serological weak D samples, 40 were genomic weak Ds and four had a partial D genotype. Six weak D and two partial D samples were confirmed at the Nordic Reference Laboratory for Blood Group Genotyping, Lund, Sweden. One mistyping of *RHe* as *e*- (Rhe+ phenotype), was controlled and confirmed as *RHe+* by inno-train.

Summary/Conclusions: RBC-FluoGene is reliable and user-friendly with results available within three hours. The method can be a supplement to inconclusive serological RhD-typing, and further applications may be adopted in the future.

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MOLECULAR CHARACTERIZATION OF RHD GENE IN RHD NEGATIVE INDIAN BLOOD DONORS

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Background: Rh blood group system is one of the most complex blood group systems and D antigen itself, consists of over 30 different epitopes. RhD DNA typing is complex due to presence of two highly homologous genes, *RHCE* and *RHD* and the complex polymorphism between both genes. The molecular polymorphism of the *RHCE* and *RHD* genes has been characterized in various populations but no systematic studies have yet been conducted for the Indian population
Aims: To assess genetic basis of RHD negative phenotype in Indian blood donor population.
Methods: We analyzed the presence of *RHD* gene in 200 phenotypically RHD negative blood donors using the PCR method. RHD phenotyping was done using automated blood grouping system (Qwalys 3, Diagast, France). Samples negative upon testing were further confirmed for absence of RHD antigen in AHG phase (ID-diaclon Anti –D, DiaMed, Switzerland). *RHD* genotyping was done using three primer sets designed for exons 4, exon 10 and one set for identification of *RHDΨ* gene between int 3 and int 4. Sample from known RHD positive donor was included in every batch as control. Amplified PCR products were analyzed by gel-electrophoresis (XY Loper, Uvitec, Cambridge) and confirmed by nucleotide sequencing (ABI 3730 xl 96 capillary system).
Results: No PCR product was found in 195/200 (97.5%) of study samples indicating homozygous gene deletion. Of the 5/200 (2.5%) showing RHD gene polymorphisms, 4/200 (2%) were positive for presence of exon 10 only, indicating the presence of RHD-CE-D hybrid gene. *RHDΨ* gene was not detected in any of the samples tested. One sample from 24 year old male, showed presence of all three tested regions, and was negative for *RHDΨ* gene, thereby indicating the presence of inactivating mutation in *RHD gene*.
Summary/Conclusions: RHD gene deletion was identified as most common cause of D –ve phenotype and RHD-CE-D hybrid gene was responsible in 2% of cases. However, *RHDΨ* gene, reported to be present in up to 39% of various ethnic populations from across the world was not identified in Indian blood donors. Since, all 04 cases showed presence of only exon 10 and no exon 4, it indicates that these samples may belong to either D VI or DFR category of partial D variant. However, further confirmation needs to be done

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Abstract has been withdrawn.

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MOLECULAR ANALYSIS OF RHD GENE IN IRANIAN BLOOD DONORS WITH D-NEGATIVE PHENOTYPE

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Background: The Rh blood group system is one of the main causes of hemolytic disease of the fetus and newborn and transfusion reactions. The *RHD* gene is highly polymorphic with more than 200 aberrant alleles. Routine serology cannot discriminate some of these alleles from normal D antigen.
Aims: The aim of this research was to characterize *RHD* alleles among serologically D-negative blood donors of Tehran, Iran.
Methods: Samples of 405 blood donors who were typed as D-negative in routine serology were analyzed. RhCE phenotypes of samples were tested serologically. SSP-PCR reactions were performed with *RHD* exon-specific polymorphisms to determine the partial or complete presence of the gene. Point mutations were detected by direct sequencing.
Results: More than 98% (397/405) of samples were negative for all RHD-specific polymorphisms, indicating deletion of the whole gene. Eight samples retained some or all sequences of the RHD, all with *Ce* haplotype, of which two determined as hybrid alleles and six characterized to harbor novel and reported point mutations.
Summary/Conclusions: D-negative phenotype is the result of *RHD* deletion in most of our population. This is compatible with the results reported for Caucasian. A novel mutation was detected suggesting the diversity of *RHD* alleles.

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ABO*A AND RHD VARIANTS IN RUSSIANS

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Background: In clinical transfusions antigens of ABO and Rhesus systems are the most relevant, and the D antigen of the Rhesus blood group system (BGS) is more important. There are more than 100 alleles encode the glycosyltransferases in ABO-system. Approximately 1%>2% of Caucasians show weak expression of D antigen, which is caused by various types of mutations in the *RHD* gene. There are three D antigen variants in RH system: weak D, partial D and Del. The people know over 200 *RHD* alleles encoding partial and weak D. It's essential to know D variant distribution in each population in order to define whether a patient has a high or low probability to produce anti-D alloimmune antibodies during erythrocyte's transfusion therapy.
Aims: to determine the distribution of A and D antigen variants in Russians.
Methods: Agglutination tests with anti-A, anti-B, anti-AB Moabs. D antigen was typed with 5 serological methods saline and indirect antiglobulin tests. The blood samples were serotyped for Rh C/c and Rh E/e antigens by corresponding Moabs (Hematolog, Russia). DNA extraction was followed by commercial SSP-PCR kits with primers for typing *ABO, RHD, RHCE,* 11 weak D types, partial D (BAG, Germany). A mutation in RHD (23 cases) was detected by direct Taq cycle-sequencing, computer based sequence analysis and comparison with reference sequences from Genbank™.
Results: In 2014–2016 years we revealed low expression of A-antigen in 318 (5.16%) and RhD antigen in 156 (2.53%) persons from 6,165 Russians by serological tests. 18 samples with weak A were genotyped. 78 people have been investigated due to diminished D expression, discordant results with different anti-D reagents, or D negative samples with atypical Rh phenotype. More 5 people were investigated on *RHD* genes. Genotyping samples with weak A revealed 12 people with A2 and A2B antigens, in two cases – phenotype was A2B, but genotype A1B1, Ael (2) and Ax (2). Genotyping 78 people revealed seven weak D types in 76 persons: weak D type 3 (51.3%; n = 39; phenotypes – C+c+E-e+ in 36 cases, C+c-E-e+ in two and atypical one C-c+E-e+) and weak D type 1 (27.6%; n = 21; phenotypes – C+c+E-e+) were the most frequent. Weak D type 2 was detected in 13.2% (n = 10; phenotypes – C-c+E+e+), weak D type 15 – in 3.95% (n = 3; phenotypes – one C-c+E+e+ and atypical two C+c-E-e+, C+c+E-e+), weak D types 4.2 (DAR; phenotype C-c+E-e+), weak D type 6 (phenotype C+c+E-e+) and weak D type 67 (by sequencing) (phenotype C-c+E+e+) – each in one person (1.3% accordingly). Two cases with RhCe and RhcE phenotype were D-negative in all serological methods, and only molecular method allowed identifying weak D type 15. More two samples with diminished D expression were partial D: variant DNB with phenotype C+c-E-e+ and DVII with phenotype C+c-E+e+. Three erythrocyte's samples were identified as *RH19, RH31 and RH32* with strong expression of D antigen. Two erythrocyte's samples had true D-negative phenotype: one – RHD_{Ψ} with phenotype C-c+E-e+, and first in Russia we found regulatory type Rh_{null} with predicted phenotype D+C+c+E-e+ in woman with meningioma. *RHAG* sequencing (A. Doescher, Germany) shows polymorphism in exon 4 of the *RHAG* gene at position 571 (C > T) which leads to an earlier Stop-codon (amino acid position 191 [R191]) and the additional mutation at position 724 of exon 5 (G > A).
Summary/Conclusions: The distribution of A and D variants in Russians was identified. These data may help to improve the transfusion strategy of people with such variants

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DROPLET DIGITAL™ PCR AS A NEW TOOL FOR THE QUANTIFICATION OF RHD AND RHCE IN SAMPLES WITH WEAK RHCE REACTIONS IN SEROLOGICAL TESTING AND WILDTYPE SEQUENCING RESULTS

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Background: Weak reactions with certain antisera are common in serological Rh-testing. A large number of genetic variants is known as molecular background for these problems. Nevertheless, some samples, especially with weak RhC reaction patterns, remained unresolved with classical molecular typing methods.
Aims: We developed a droplet digital™ PCR (ddPCR) assay for co-amplification of *RHD*- and *RHCE* specific sequences in exon 3 to 6 to further examine these samples and to compare the results with quantitative real-time PCR findings.
Methods: Nine samples with unresolved genotypes were tested in dd PCR (QX 200 Bio Rad) and quantitative real-time PCR (7900HT Applied Biosystems).
1. The ddPCR assay was performed in a mix of ddPCR buffer, 900 nM primer, 300 nM probes and samples diluted 100-fold. Amplification of *RHD*- and *RHCE*-specific sequences was performed in the same tube and compared to known DD- and Dd controls.
2. Quantitative real-time PCR: Four different DNA concentrations per sample were used for co-amplification of *RHD* and *RHCE* in the same tube. Threshold cycles (Ct) were manually set and the difference between *RHD*- and *RHCE*-specific Ct values was calculated as ΔCt.
Results: Four samples with weak RhC phenotypes were used to assess the reliability of the method. A masked *RHCE* hybrid gene was detected in each sample. Three samples with weak reactions in RhC/RhD typing showed a decreased amount of the CDe haplotype. Examination of the RhC-negative sample from a newborn of a homozygous RhC mother revealed a -D- haplotype. No differences were observed between dd- and quantitative real-time PCR.
Summary/Conclusions: The quantification of *RHD* compared to *RHCE* exons 3 to 6 provides additional information of the molecular genetic background in samples with weak phenotypes and presumably normal genotypes. If samples for RNA extraction and haplotype-specific sequencing of cDNA are not available, the quantification of *RHD* and *RHCE* offer an efficient alternative to detect masked hybrids and reduced amounts of one haplotype. Droplet digital PCR introduces a reliable and cost-effective alternative to quantitative real-time PCR in this setting.

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STUDY ON POLYMORPHISM OF SMP1 GENE IN CHINESE HANS AND TIBETANS KEYWORDS: SMP1 GENE; HAN NATIONALITY; TIBETAN NATIONALITY; POLYMORPHISM;

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Background: The non-coding region of Rh gene mainly included richly polymorphic promoter, intron, flanking sequence and spacer, which of above regions were important for RH gene to regulate gene expression. The spacer between RHD and RHCE gene involves the downstream box and a SMP1 gene. 3, UTR in exon 7 of the SMP1 gene known as TMEM50A (transmembrane protein 50A) was partial overlap with exon 10 of RHCE gene. Speculated from the location that the polymorphism of SMP1 gene is closely linked with special RH haplotype, and relatively functional mutation may be related to selective pressure of special RH haplotype. Recent researches focus on coding region rather than on non-coding region, furthermore, there are few studies on SMP1 gene, and researches on Tibetan SMP1 gene has not been reported.
Comparative study on SMP1 gene between Chinese Hans and Tibetans was helpful to provide insight into RH gene structure and ethnic genetic differences, to explore the functional mechanism of RH gene in evolution, recombination and mutation, as well as to reveal genetic background of RHD and RHCE polymorphism.
Aims: To research comparatively on gene structure and polymorphism of SMP1 gene between RHD and RHCE gene in Chinese Hans and Tibetans, and to establish specific detection for SMP1 gene. To study comparatively on ethnic background of *SMP1* polymorphism.
Methods:
1. SMP1 gene were amplified by polymerase chain reaction (PCR) with specific primers designed according to reported RH gene (BN000065.1 and NC_000001) and SMP1 gene (NT_004610.19) sequences from GenBank, and amplicons were sequenced after being purified.
2. Sequence and structure of SMP1 gene were analyzed with DNAMAN5.2.9 software and REPEATMASKER WEB SERVER (http://ftp.genome.washington.edu/cgi-bin/RepeatMasker).
3. 1351T>C polymorphism of SMP1 gene was detected with PCR-SSP whose primers were directed to 1351T>C polymorphism.
Results:
1. The whole length of SMP1 gene was 24,066 bp in Hans and Tibetans, and composed of 7 exons and 6 introns. The length of exon 1–7 of SMP1 gene was 159 bp, 106 bp, 113 bp, 68 bp, 93 bp, 61 bp and 1702 bp respectively, while the length of intron 1–7 of SMP1 gene was 2017 bp, 2381 bp, 8553 bp, 1188 bp, 3818 bp and 3807 bp respectively.
2. The SMP1 gene comprised A: 6631 bp (27.5%), T: 7390 bp (30.7%), C: 5026 bp (20.9%), G: 5019 bp (20.9%), and 3 A-T rich regions as well as 34 Alu(SINE/Alu) sequence were identified.
3. Polymorphism was not found in exon 1–6 of SMP1 gene, while 1351T>C and 1726G>A polymorphism was found in exon7.
4. There existed significantly different ($P < 0.05$) between the Hans and the Tibetans in the distribution of 1351T>C polymorphism.
Summary/Conclusions:
1. The SMP1 gene showed conserved structure in Hans and Tibetans. Polymorphism was not found in exon 1–6 of SMP1 gene, while 1351T>C and 1726G>A polymorphism was detected in exon7.
2. The methods for detecting the exon of SMP1 gene and 1351T>C polymorphism were set up successfully and that could be used to research Chinese SMP1 gene structure. There existed significantly different between the Hans and Tibetans in the 1351T>C polymorphism.

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MLPA MOLECULAR ANALYSES OF RHD AND RHCE VARIANT ALLELES IN BRAZILIAN TRANSFUSED PATIENTS AND BLOOD DONORS WITH TYPING AND SEROLOGIC PROBLEMS

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Background: Due to complexity of the Rh system and its more than 280 variants alleles, the serologic resolution of the complex cases has been increasingly complicated. Therefore, the genotyping has been used to solve these problems.
Aims: To Identify *RHD* and *RHCE* variant alleles in blood donors and patients with typing and serologic problems using MLPA assay.
Methods: Donors (n = 56) and patients (n = 54) were typed for RhD antigen by hemagglutination (DG-Gel ABO/RhD, Grifols). The antibody identification of patients was done by gel technique (DG-Gel, Grifols). All samples with atypical RhD typing results or antibodies in the presence of the corresponding RhD and/or RhCE antigens were genotyped by Multiplex Ligation-dependent Probe Amplification (MLPA) assay (MRC Holland) which is able to distinguish 51 *RHD* and 13 *RHCE* variant alleles. For another 28 *RHD* and 4 *RHCE* variant alleles the methodology can identify the main type but does not discriminate the subtypes.
Results: Considering the patients population we found 30/54 (55.5%) *RHD* variant alleles: *RHD*DAU0,1,2,3,6,7/RHD*DV.1* (n = 1), *RHD*DIIIa-CE(3–7)-D/RHD*DAU0,1,2,3,6,7* (n = 1), *RHD*DIIIa-CE(3–7)-D/DIIIa* (n = 1), *RHD*weak D type 1* (n = 4), *RHD*weak D type 33/DAU4* (n = 1), *RHD*weak D type 4.0 or 4.1* (n = 3), *RHD*Ψ* (n = 1), *RHD*DIII.4 or DIVa.2/DAR1 (n = 1). RHD*DAR1* (n = 4), *RHD*DII* or *DIV.4* (n = 1), *RHD*DIII.4* or *DIVa.2* (n = 1), *RHD*DIIIa-CE(3–7)-D/CE(3–9)-D* (n = 1), *RHD*DIIIa-CE(3–7)-D/DAR1* (n = 1), *RHD*DIIIa-CE(3–7)-D/weak D type 1* (n = 1), *RHD*weak D type 2* (n = 1), *RHD*Ψ/weak D type 1* (n = 1), *RHD*DAU0,1,2,3,6,7(n = 3)*, *RHD*DIIIa* (n = 1), *RHD*DIIIa-CE(3–7)-D* (n = 1), *RHD*Ψ/DIII.3 (n = 1)*. Furthermore, we found 25/54 *(46.3%) RHCE* variant alleles: *RHCE*ce48c (n = 6), RHCE*ceVS* (n = 9), *RHCE*ceVS/ceVS* (n = 3), *RHCE*ce48C-D (9)-ce* (n = 1), *RHCE*ceAR or ceEK/ceVS* (n = 2), *RHCE*ce48c/ceAR* or *ceEK* (n = 3), *RHCE*Ce800A* (n = 1), *RHCE*ce-D(9)-ce* (n = 1). Eight out of 30 patients with *RHD* variant alleles showed antibodies associated with RhD antigen and 16/30 presented weak or partial expression of RhD antigen. Considering the patients with *RHCE* variant alleles, 4/25 showed antibodies associated with RhCE antigens. Twenty three out of 54 patients showed *RHCE* and *RHD* variant alleles concomitantly. In the blood donors population, we found samples with weak D and partial D expression. 41/56 (70.6%) *RHD* variant alleles: *RHD*DAR1 (n = 9), RHD*DAU5 (n = 2), RHD*weak partial D type 15 (N = 1), RHD*weak D type 4.0 or 4.1 (N = 1), RHD*DAR1/DIIIa-CE(3–7)-D (n = 2), RHD*DAR1/DNB (n = 1); RHD*weak D type 1 (N = 4),*

*RHD*weak D type 2 (N = 14), RHD*weak D type 3 (N = 4), RHD*weak D type 2/DAR1 (N = 1), RHD*weak D type 3/DAR1 (N = 1); RHD*y/DAR1 (N = 1).* And 20/56 (34.5%) *RHCE* variant alleles: *RHCE*ceVS (n = 4), RHCE*ceAR or ceEK/ce-D(9)-c (n = 1); RHCE*ce48c (n = 3), RHCE*ceAR or ceEK (n = 12).* Nineteen out of 56 donors showed *RHCE* and *RHD* variant alleles concomitantly.

Summary/Conclusions: The MLPA method showed to be reliable to predict the presence, absence and the copy number variation of *RHD* e *RHCE*. However, samples with undetermined results or detected with only the main type, the *RHD* and *RHCE* genes should be sequenced. The results showed the importance of a comprehensive molecular investigation in serologic complex cases.

P-498

SCREENING OF CLINICALLY RELEVANT RHD AND RHCE VARIANT ALLELES IN BRAZILIAN BLOOD DONORS AND PATIENTS WITH HAEMATOLOGICAL DISEASES USING MULTIPLEX LIGATION-DEPENDENT PROBE AMPLIFICATION (MLPA)

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Background: The Rh system is one of the most complex and immunogenic blood group systems, and wide genetic diversity exists in *RHD* and *RHCE* resulting in almost 300 *RH* variant alleles. *RHD* and *RHCE* variant alleles predicting partial antigens are prevalent in individuals of African origin, and this contribute to the development of clinically significant Rh alloantibodies mainly in SCD patients. *RH* genotyping has been increasingly used in the resolution of complex cases in immunohematology, as well as in the screening of *RH* matched blood donors for African descent patients. The multiplex ligation-dependent probe amplification (MLPA) technology is a convenient single assay which can determine clinically relevant *RHD* and *RHCE* alleles and *RHD* zygosity simultaneously.

Aims: To evaluate the MLPA genotyping assay in the search for clinically relevant *RHD* and *RHCE* variant alleles and to determine the distribution and prevalence of RH alleles in Brazilian blood donors and in patients with haematological diseases.

Methods: *RH* genotypes were determined in DNA samples from 158 patients (101 with SCD, 14 with MDS, 17 with AML and 26 with AHA) and 198 random Brazilian blood donors. Molecular analysis was performed with the Blood-MLPA assay (Probemix P401, P402, P403; MRC Holland) using a thermal cycler (Veriti, Applied Biosystems) and a capillary electrophoresis equipment (3130XL, Applied Biosystems). *RHD* and/or *RHCE* exon-specific sequencing was performed to discriminate RHD and RHCE subtypes not distinguished by MLPA.

Results: We observed that 29/198 donors (14.6%) and 26/158 patients (16.5%) presented *RHD* variant alleles. The most prevalent *RHD* variant allele was *RHD*DAU0*, present in 10/198 (5.1%) donors and in 15/158 (9.5%) patients. Clinically relevant *RHCE* alleles were found in 25/198 (12.6%) donors and in 39/158 (24.7%) patients. *RHCE*ceVS.01* was the most prevalent *RHCE* variant allele and was found in 14/198 (7.1%) donors and in 31/158 (19.6%) patients. The comparative analyses of *RHCE*ceVS.01* allele frequencies between donors ($f = 0.0354$) and patients ($f = 0.1076$) were statistically significant (P = 0.0001). We found *RHCE* variant alleles inherited with *RHD* variant alleles in 13/198 (6.6%) donors and in 12/158 (7.6%) patients. The following *RHD* and *RHCE* alleles combinations were found in the studied populations: *RHD*DAU0* with *RHCE*ceMO* and *ceVS.01*; *RHD*DAU3* and *DAU5* with *RHD*ceVS.01*; *RHD*DIVa* with *RHCE*ceTI*; *RHD*weak D type 4.2.2* with *RHCE*ceAR*; *RHD*weak D type 4.0* with *RHCE*ceVS.02*, *ceVS.04* and *ce(48C, 105T, 733G, 744C, 1025T)*; *RHD*DIIIa* with *RHCE*ceVS.03*; *RHD*DIIIaCE(4–7)-D* with *RHCE*ceVS.03* and *RHD*DIII.04* with *RHCE*ceVS.02*. Eight donors and nine patients (two non-SCD) had *RHD* genotypes predicting partial D antigens. Fifteen donors and 19 patients (six non-SCD) had *RHCE* genotypes coding partial c and partial e antigens and/or lacking high prevalence RhCE antigens. Five patients (3.2%) and one (0.5%) donor had inherited RHCE variant alleles in homozygous or compound heterozygous state.

Summary/Conclusions: The Blood-MLPA genotyping assay is a reliable method to determine many clinically relevant *RHD* and *RHCE* variant alleles in Brazilians. *RHD*DAU0* and *RHCE*ceVS.01* were the most prevalent variant alleles found in Brazilians. Finally, it is important to develop new strategies targeting donors with RhCE variants and to consider *RH* variant alleles in non-SCD patients.

P-499

MOLECULAR RHD SCREENING IN SEROLOGICALLY D-NEGATIVE DONORS

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Background: A non-negligible fraction of RhC^+ and RhE^+ donors, serologically classified as RhD-negative, have been reported to have new *RHD* mutations or *DEL* alleles (1:350 in Europe), that due to the low number of antigenic sites in the RhD protein are not detected with the traditional serological methods. The identification of these genetic variants requires the adoption of modern strategies of *RHD* molecular screening.

Aims: This is a retrospective study aimed at investigating the *RHD* genotypes in a population of 100 RhC^+ or RhE^+ Caucasian donors, serologically classified as D-negative.

Methods: RhD typing was carried out with the use of 2 different anti-D IgM clones [Clone 1 (DVI+): LDM1 + ESD1M; Clone 2 (DVI-): RUM-1, TH28] in microplate direct agglutination tests (Galileo-Neo, Immucor) and 2 different anti-D IgG clones [Clone 1: MS26; Clone 2: D415 1E4] in solid phase for the determination of weak expression of D antigens. All the RhD-negative donors, 79 RhC^+ (79%) and 21 RhE^+ (21%), were genotyped by using the RHD BeadChip kit (Immucor/BioArray Solutions) and/or SSP-PCR assays (BAGene and Inno-Train). Donors showing discordant results between serology (D-negative) and molecular analyses (RHD "wild type" or "full length") were subjected to further study by direct sequencing of the whole *RHD* coding region.

Results: With the RHD BeadChip kit and/or SSP-PCR analyses, 95% of the donors showed a complete deletion at the *RHD* locus. In 5 cases (5%) a status with a "full length" *RHD* was observed. *RHD* direct sequencing revealed that 2 cases had single nucleotide deletions in exon 5 (c.697delG and c.702delG, respectively), responsible of frameshift alterations that led to the onset of a stop codon at residue 245 (V245X). A donor with a drop-out of exon 9 markers and another donor with *RHD* genotyped as "wild type" are still under investigation with sequencing analyses. A fifth case, with an *RHD* "full length" and RhC^+, was found to have an IVS3 + 1G>A (DEL) (*RHD*208*) allele with both the RHD BeadChip and SSP-PCR tests (Ready-Gene D AddOn, Inno-Train). This donor was re-classified as RhD-positive.

Summary/Conclusions: The present study puts in evidence 2 new *RHD* mutations, leading to a D-negative phenotype, alternative to the complete deletion of the *RHD* locus which is common in the D-negative subjects of Caucasian ancestry. In addition, we identified an IVS3 + 1G>A(DEL) variant that may induce anti-D immunization in the recipient. This had never been found in our serology routine, that had always identified other DEL variants like the "M295I" (or Weak D type 11). Besides the decreased number of antigenic sites, the IVS3 + 1G>A(DEL) variant is characterized by a splicing alteration that may cause the lack of some D-specific epitopes. D-negative donors with RhC^+ and RhE^+ phenotypes are reported to mask DEL alleles in serological tests with a frequency of 1:51 and 1:344, respectively. Hence, the choice of routine antisera to be used in the determination of D types with very weak reactivity is crucial. Finally, the use of molecular methods is highly recommended to better characterize those donors who are "apparently" D-negative, in order to guarantee transfusion safety.

P-500

A DFR SEROLOGICAL PATTERN CAUSED BY A NOVEL MISSENSE MUTATION

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Background: The identification of aberrant expressions of the D antigen is critical in blood banks to optimize transfusion compatibility and in obstetrics to define strategies for medical intervention in pregnant women. In most cases, serological determinations are inconclusive and in this context the study at DNA level is appropriate to overcome limitations.

Aims: The aim of this study was to characterize the molecular background of two D variant (D^{var}) samples referred to our laboratory for *RHD* genotyping.

Methods: Commercially available monoclonal reagents were used to evaluate the Rh phenotype (D, C, c, E, e) of donors' red blood cells through standard tube

hemagglutination techniques including the indirect antiglobulin test (IAT). Particularly, D antigen status was examined with two blended IgM/IgG anti-Ds (clones TH-28 + MS-26; Wiener Lab, Rosario, Argentina and clones P3x61 + P3x21223b10 + P3x290 + P3x35; Diagast, Loos, France), and three IgM anti-Ds (clone MS-201, clone RUM-1 and clones LDM1 + ESD1M; Rediar, Buenos Aires, Argentina). Partial D phenotypes were studied using the ID-Partial RhD Typing Set (DiaMed, Cressier, Switzerland). A modified salting-out method was used to isolate genomic DNA. *RHD* zygosity status was analysed by a PCR-RFLP method. Weak D types were evaluated by allele-specific PCRs. Samples were subjected to a *RHD* exon scanning procedure based on PCR-SSP to analyse multiple *RHD* exon polymorphisms. Direct automated sequencing on PCR products of the 10 *RHD* exons was also performed.

Results: Direct tube testing showed a weak reactivity with all anti-D reagents used. IAT enhanced the blended anti-Ds hemagglutination. The Rh phenotype was D^{var}, C+, c-, E-, e+ for proband #1 and D^{var}, C+, c+, E-, e+ for proband #2. Both samples showed a clear DFR reaction pattern when tested with the monoclonal anti-D reagents of the ID-Partial RhD Typing Set. One hybrid *Rhesus box* was detected in each sample suggesting a hemizygous status for the *RHD* locus. Neither allele-specific PCRs nor exon-scanning analyses showed modifications in the polymorphisms studied. Interestingly, genomic DNA sequencing revealed a new *RHD* variant characterized by the point mutation 325A>G in *RHD* exon 2.

Summary/Conclusions: We report a novel *RHD* allele with a missense mutation responsible for the amino acidic change Thr109Ala, predicted to be in the extracellular boundary of the fourth transmembrane segment of the RhD protein. The ID-Partial RhD Typing Set indicated a DFR phenotype in both samples. However, DNA sequencing showed a different genetic background from the DFR-1, DFR-2, DFR-3, DFR-4 and DFR-5 alleles described so far. While these variants result from hybrid structures involving *RHD* exon 4 (and exon 3 in DFR-5), a point mutation in *RHD* exon 2 is responsible for the variants reported in this work. Serologic and molecular results suggest a genetic association *in cis* between this new *RHD* variant and the *RHCe* allele. Our findings show that molecular studies are a necessary complement for characterizing samples with variant D phenotypes. *RHD* genotyping may contribute for clinical decision making in transfusion and to provide appropriate recommendations for anti-D prophylaxis in pregnancies.

P-501

AN RHD*C.1066 INS A MUTATION INDUCES PARTIAL D REACTIVITY

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Background: A patient's Rhesus D blood group could not be clearly determined by serological testing by the submitting laboratory. A weak D or partial D was supposed but the reactivity pattern could not be assigned to a known weak D or partial D.

Aims: The patient's Rhesus D blood group should be determined unambiguously by serological as well as molecular methods.

Methods: The patient's RBC were screened applying haemagglutination with two IgM monoclonal Anti-D (ID card DiaClon ABO/D+DAT; DiaMed, Cressier, Switzerland) with automatic reading (IH-500, Bio-Rad, Munich, Germany). Reactivity was further differentiated applying D screen for D category in a gel card system (Diagast, Loos, France). Different commercial PCR-SSP tests were applied: RH-Type, (BAG Health Care Lich, Germany), RBC-Ready Gene CDE, (Innotrain, Kronberg, Germany), RBC-Ready Gene D weak (Innotrain), Weak D-Type (BAG Health Care), RBC-Ready Gene D AddOn (Innotrain) and Partial D-Type (BAG Health Care). DNA sequencing of the ten *RHD* exons including short flanking intron sequences were amplified in a gene specific manner by use of published primer sequences. Cycle sequencing was performed with the Big Dye Terminator v3.1 chemistry (ABI, Weiterstadt, Germany) followed by electrophoretic separation in an ABI Prism 310 DNA analyser. Determined DNA sequences were aligned to published reference sequences.

Results: The patient's RBC did not or only very weakly react with the IgM monoclonal Anti-D clones LHM50/3, TH-28, RUM-1, LHM59/20, 175-2, P3x61, HM16, P3x21211F1 and P3x21223B10, but were clearly positive with the IgG clones HM16, P3x35, P3x241, P3x249 and P3x290. PCR-SSP assays assigned a CcDee type without any hint to a weak D or partial D. DNA sequencing demonstrated an insert of an additional Adenin at position 1066 of the *RHD* gene (*RHD*c.1066insA*). This induced a frame shift within exon 7, leading to a premature stop at codon 391 instead of codon 418. Amino acid changes within the sixth extracellular loop and abolishment of the intracellular carboxy terminus of the RhD glycoprotein thus seems to have induced differential binding of the monoclonal IgM anti-D to the RhD epitopes. The new mutation was submitted to GenBank under the accession number KY659317.

Summary/Conclusions: A new *RHD*c.1066insA* mutation induced a partial D phenotype in a patient.

P-502

EXPRESSION OF D EPITOPES IN CARRIERS OF RHCE VARIANTS: DETECTION OF THE CESL VARIANT IN RHD NEGATIVE SPANISH BLOOD DONORS

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Background: Expression of D epitopes in *RHCE* variants was first described in 1996, when the molecular basis of the R_o^{Har} phenotype was established. The *DHAR* variant associated with this phenotype is a hybrid allele *RHCE-D(5)-CE*, in which exon 5 of the *RHCE* gene is replaced by its *RHD* counterpart. The observation of anomalous or discrepant results with anti-D MoAbs has allowed the identification of other *RHCE* variants associated with D epitope expression in *RHD* negative individuals. These include *RHCE* variants whose alterations do not involve the substitution of amino acid residues by D-specific amino acids.

Aims: The aim of the present study was to characterize the molecular basis responsible for a clearly atypical pattern of reactivity with anti-D MoAbs observed in two unrelated blood donors during routine RhD typing of their blood donations.

Methods: Both donors showed discrepant results during RhD typing at the Blood Center. The only reagent with a positive result was anti-D IgG+IgM (Pelikloon, Sanquin) in the PK7300 analyzer (with bromelin-treated erythrocytes). D antigen expression was further analyzed with additional anti-D reagents: TOTEM (Diagast), anti-D NOVACLONE (IgM+IgG) and ImmuClone Fast IgM (Immucor) and with the D-screen panel (Diagast). For the molecular study, DNA samples from the two blood donors and available relatives were extracted using the QIASymphony extractor. *RHD* gene exon scanning and *RHCE* genotyping was performed by PCR-SSP. All *RHCE* exons were sequenced and analyzed using an ABI PRISM 3130 Genetic Analyzer and Accelrys Gene 2.0 software for alignment with the *RHCE* sequence (Ref. Seq: NG_009208.3).

Results: In the extended serological study of red blood cells from these two donors, a very weak positivity by direct agglutination was detected only with the TOTEM anti-D reagent, which was negative in antiglobulin phase. No agglutination was observed with any of the additional anti-D reagents tested. Molecular analysis of the *RH* locus revealed the donors were *RHD* negative, since no *RHD* exon amplification was obtained using *RHD*-specific primers. The *RHCE* genotype showed the presence of two *RHCE*ce* alleles, whose sequence was analyzed because of a suspected variant. A heterozygous C>T change at position 365 (365C>T) was detected in exon 3, which causes the amino acid change Ser122Leu in the fourth transmembrane segment. This change has been previously described in the *RHCE*ceSL* variant, detected in the Swiss population (Kanton Bern). A family study has been conducted in one of the new cases identified in Spain and the father was found to carry the same mutation.

Summary/Conclusions: The binding of some anti-D reagents to this *ceSL* variant is not well understood but it might be related to a conformational change in the RHce protein due to the Ser122Leu substitution in the fourth transmembrane segment, which could emulate some D epitope. The finding of two unrelated cases of the *RHCE*ceSL* variant in the Spanish blood donor population shows that this mutation is not confined to Kanton Bern in Switzerland. The potential sensitization of D-negative receptors will be investigated.

P-503

A REVIEW OF RHCE INFERRED PHENOTYPES AND RED CELL GENOTYPING DATA IN DONORS SEROLOGICALLY CONFIRMED AS NEGATIVE FOR THE HIGH FREQUENCY ANTIGEN RH:34

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Background: The HrB(Rh:34) and hrB(Rh:31) antigens were defined in the literature in the 1970's after tests confirming the presence of the corresponding antibodies were found in the serum of a black South African woman. Rh34 is a high frequency antigen that is significant in South Africa as it is negative or absent in <0.2% RhD+/RhD- black donors thus defining them as rare donors. Anti-Rh34 is also a clinically significant antibody capable of causing Haemolytic Disease of the

Newborn. The South African National Blood Service (SANBS) performs red cell genotyping on selected rare donors on the South African Rare Donor File as serology on its own is limited due to lack of rare expensive antisera. The IDCORE^{XT} assay tests for the hrB (Rh:31), however samples that test negative for Rh:31 gene may also be negative for HrB (Rh:34) and this could be a surrogate test for detecting potential Rh:-34 donors.

Aims: To review the RHCE phenotypes and red cell genotyping on donors serologically confirmed as Rh:-34 to identify phenotypic and genotypic result patterns that can be used as triggers when screening for Rh:-34 rare donors.

Methods: Samples from four serologically confirmed Rh:-34 black male donors were sent for DNA extraction on the Maxwell AS2000 instrument. Red cell genotyping for the Rh:31 gene was completed using the IDCORE^{XT} kit on the Luminex platform. DNA was outsourced for DNA sequencing. The molecular genotyping results and inferred phenotypes of the 4 donors were analysed and reviewed.

Results: Two donors (donor 1 and 2) were RHD- and displayed partial C, E-, partial c, partial e, VS+V- and Rh:-31 phenotypes. The third donor was partial RHD+ and had C-, E-, partial c, partial e, VS+V- and Rh:-31. The fourth donor was RHD+, partial C, E+, partial e, VS+V- and Rh:-31. At a molecular level, the partial C antigen is encoded by alleles RHD*DIIIa-CE(3–7)-D which was present in all donors except the third donor which was C(Rh:02) negative. A silent polymorphism c.609A was identified on exon 4 of the RHCE gene in donor 2 however this has no impact on the predicted phenotype which is the same as compared to donor 1. The Rh:-31 phenotype is usually linked to RHD*DIIIa or hybrid RHD*DIIIa-CE-(3–7)-D and with homozygous 48C, 733G and 1006T polymorphisms which is present in all except the fourth donor where it is only at one allele. The fourth donor has an E+ phenotype with weak detection of Rh:-34 serologically. The third donor is an example of the RHD*IIIa hybrid with a c.150C polymorphism.

Summary/Conclusions: From the results above, the serological triggers identified will be partial or weak agglutination reactions with the C, e, and c phenotypes. VS will always be positive with V negative. At a molecular level, the RHD*DIIIa hybrids are evident and literature has supported that the presence of homozygous 733C>G in exon 5 and 1006G>T in exon 7 of the RHCE gene could replace serological phenotyping for Rh:-34. A strategic objective at SANBS is to introduce next generation sequencing to perform genomic analysis of Rh genes within the next 5 years.

P-504

A SIMPLE STRATEGY TO SCREEN THE RARE (C) CES HAPLOTYPE IN BLOOD DONORS

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Background: The *(C)ce^S* haplotype, mainly found in Black individuals typed as D-C+, rises from two altered hybrid genes *RHD-CE-D ^{S}* segregated with *ce^S* allele. Both genes, which differ in *RHD* sequence, show the D-, partial C, weak e, VS+V-, hr^B- and Hr^B-phenotype. This phenotype, mostly found in sickle cell disease (SCD) patients, is considered rare and difficult to be identified.

Aims: Aiming to give a better transfusion support to these patients, we propose a simple strategy to detect hr^B- and Hr^B- blood donors in a population of high degree of admixture.

Methods: We selected 68 DNA samples from donors previously typed as D-C+. These samples were submitted to a multiplex PCR which amplifies intron 4 and exon 7 of *RHD* and also the *RHDΨ*. Samples with negative results were submitted to a specific *RHD* exon 9 PCR, and those with positive results were genotyped (SSP-PCR) for 733C>G and 1006G>T polymorphisms in *RHCE*, characterizing the *(C)ce^S* haplotype.

Results: *RHD* intron 4 and exon 7 were not amplified in all of the 68 donors D-C+ tested, while 34 (50%) of them were *RHD* exon 9 positive. Analysis of *RHCE* gene on those 34 donors showed that all samples presented the *(C)ce^S* haplotype, being 33 (48.5%) heterozygous and 1 (1.47%) homozygous. When we analyzed the ethnic background of the donors studied, we observed that only 21.2% of the donors with the *(C)ce^S* haplotype were self-declared as Afrodescendants, while the remaining donors were self-declared as Caucasians.

Summary/Conclusions: Our results showed a high frequency of the *(C)ce^S* haplotype in the studied population. Although the presence of *(C)ce^S* haplotype is related to Afrodescendant individuals, in an admixed population, as the Brazilian population, the screening cannot be restricted to Black donors, since 78.8% of the donors carrying this haplotype were self-declared as Caucasians. The strategy here proposed is able to detect hr^B- and Hr^B- phenotypes using simple and low cost molecular assays and can be implemented to screen rare blood donors in order to perform a better matching for patients with SCD.

P-505

A NEW RHCE*01 ALLELE HARBORING THE P.TYR269STOP NONSENSE MUTATION SEEMS TO ABOLISH THE RHCE EXPRESSION, AND WHEN ASSOCIATED WITH THE RHD*01N.01 ALLELE MAY BE RESPONSIBLE FOR THE RHNULL PHENOTYPE

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Background: The Rh blood group system currently comprises 54 antigens which are encoded by the *RH* locus. It is composed of two highly homologous genes *RHD* and *RHCE* which are located on chromosome 1. Both genes are composed of 10 exons, which are distributed in an opposite orientation. Rh$_{null}$ (Rh29-) individuals are defined as not expressing any Rh antigens and are classified in 2 types. The regulator type, which is more common, is defined as an alteration in the *RHAG* gene. It encodes a glycoprotein which is essential for the expression of the Rh antigens. The amorph type is defined as an alteration of the *RHCE* gene. Its molecular basis is the silencing of one of the four following alleles when associated with a deleted *RHD* allele: *RHCE*01 – RHCE*02 – RHCE*03* or *RHCE*04*. Individuals of both types exhibit a mild clinical syndrome which is characterized by a chronic hemolytic anemia.

Aims: We report the case of an individual routinely phenotyped as D-C-E-c-e- and harboring a new mutation that seems to abolish the *RHCE* expression.

Methods: Standard hemagglutination techniques and *RHD*/*RHCE* genomic DNA sequencing with "in house" techniques were performed.

Results: The serum of a Caucasian male (84 y/o) was referred to our IRL in order to confirm his Rh$_{null}$ phenotype. His medical history was scarce. Neither previous transfusion nor type and screen were reported. His D-C-E-c-e- phenotype was confirmed with monoclonal and polyclonal reagents. The antibody screen was negative. The patient had a nephrectomy without any transfusion. The proband's parents were first-degree cousins from a remote rural area. The 10 exons of the *RHD* and *RHCE* genes were investigated. For the *RHD* gene, there was no exon amplification, indicating the presence of *RHD*01N.01* allele at a homozygous state. For the *RHCE* gene, the *RHCE*01* allele was found at a homozygous state but with a nonsense mutation c.807T>A inducing the codon change p.Tyr269stop in exon 6. Adsorption/elution using a potent anti-Rh17 (made by a D – – patient) on the proband's RBCs showed negative reactions on rr and R_1R_1 RBCs, but unexpectedly showed a very weak expression on R_2R_2 RBCs.

Summary/Conclusions: We report here a new *RHCE* allele harboring the p.Tyr269-stop nonsense mutation that seems to abolish the *RHCE* expression. The GenBank accession number is KP090269. To our knowledge, fewer than 10 silent alleles have been reported on an *RHCE*01* allele basis. The molecular background is either a deletion or a splice site mutation. Interestingly, this is the first described *RHCE*01* allele with a nonsense mutation. Unexpectedly, the eluate was very weakly positive on R_2R_2 RBCs, which led us to think that this mutation might encode a minute amount of *RHCE* molecules. Further investigation will be needed to support this hypothesis. It is extremely uncommon to find an Rh$_{null}$ amorph individual. Few cases were described in Japan, Brazil or Western Europe. A high consanguinity rate was found in the probands' families. We can suppose that Rh29- individuals might be slightly more numerous than expected in population where genetic admixture is low or a new phenomenon.

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Abstract has been withdrawn.

P-507

CHARACTERIZATION OF THE RHD BLOOD GROUP GENE IN PATIENTS WITH WEAK RHD ANTIGEN EXPRESSION

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Background: The Rh blood group system is very complex and consists of 54 blood group antigens encoded by two adjacent genes, *RHD* and *RHCE,* on chromosome 1. *RHD* encodes a multi-pass membrane protein on erythrocytes which bears the RhD blood group antigen. RhD is highly immunogenic and anti-D is the most clinically important antibody outside of the ABO system. Some individuals have variants of

RhD that are expressed more weakly than normal. These may be mainly quantitative: so-called weak D, and often arise from amino acid exchange in transmembrane and intracellular domains of the protein. Others are qualitative and lack specific RhD epitopes as determined by their pattern of reactivity with a panel of monoclonal anti-D. The D_{el} phenotype is a weakly expressed RhD variant that requires adsorption/elution techniques in order to detect the antigen. In all cases, there is a risk that an individual may be mistyped, which has consequences in blood transfusion, pregnancy and blood donation.

Aims: The purpose of this study was to characterise samples from weak RhD and apparently RhD-negative individuals in which an *RHD* gene had been identified, after exclusion of the *RHD* pseudogene (*RHD*08N.01*) and alleles *RHD*01W.1 to 5*.

Methods: We investigated twenty samples referred to our laboratory known to express a weak D or type as RhD-negative). All met the inclusion criteria described above. The ten exons of the *RHD* gene including 50 base pairs of the flanking introns were sequenced on genomic DNA.

Results: The weak D alleles, *RHD*01W.58* and *RHD*01W.92* were found in three and two individuals, respectively. In addition, seven more alleles (*RHD*01W.122*, *RHD*Weak partial 15*, *RHD*19*, *RHD*weak partial 11*, *RHD*01W.10*, *RHD* 01W.128*, *RHD*01W.78*) were identified among the samples, all previously described in individuals with weak or partial RhD phenotype. In one sample with very weak D expression, a single nonsense SNP (c.1203T>A; p.Tyr401Ter) was found. This mutation had previously been found in both D negative and Del individuals, and had been given two ISBT numbers: *RHD*01N.22* and *RHD*01EL.17*. The null allele *RHD*01N.13* (c.487_490delACAG), giving rise to a frameshift and premature stop codon was found in one RhD-negative individual. Three splice site mutations (c.IVS2-1G>A, c.IVS2-2 A>G, c.IVS7-2 A>C) were detected, all previously described in individuals expressing very weak or no D antigen. They have been assigned ISBT numbers *RHD*01N.24*, *RHD*01EL.33 and RHD*01N.81*. For the remaining three samples, no mutations in exons or adjacent introns could be detected to explain the weak expression of RhD.

Summary/Conclusions: Fourteen different alleles were found in 17 samples illustrating the great diversity in the genetic background of weak RhD and RhD-negative individuals. Up to now, over 150 *RHD* alleles giving rise to weak D and D_{el} phenotypes have been given numbers according to the ISBT standard. Correct identification of the RhD phenotype remains clinically relevant, both in the provision of the right blood to patients and in the appropriate management of the already sparse supply of RhD-negative blood units.

P-508

THE FIRST RESULTS IN GENOTYPING SEROLOGICAL WEAK FORMS OF THE D ANTIGEN AMONG BLOOD DONORS OF REPUBLIKA SRPSKA

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Background: The Rh system is very complex, polymorphous and the most significant for clinical practice, along the ABO blood group system. The D antigen is the most important antigen in the Rh system and the most immunogenic following the ABO antigens. The D antigen, which consists of a mosaic of epitopes, is determined in all the blood donors and patients. Most people are either RhD positive or RhD negative, but there is a certain number of people who have a variation of the D antigen, which are called weak D, partial D and DEL phenotypes.

Aims: The objective is to use molecular methods to determine whether blood donors in Republika Srpska (with whom a serological weak D antigen has been detected) really have the weak D antigen, partial D, a combination of these two variants or if their D antigen is normally present, but the tests used anti-D serum without avidity needed to prove the presence of this antigen in blood donors.

Methods: Blood samples were used from regular blood donors, who have been determined as persons with a weaker D antigen (based on the agglutination strength) using serological techniques, the test tube method, the microplate method and the gel method. To determine the blood groups and red blood cell/erythrocyte antigen typing, the following methods were applied: a) test tube method or agglutination in an aqueous environment, b) gel method, c) microplate method, (d) molecular determination of blood groups.

Results: Blood group samples were collected from April 2016 to February 2017 in the Institute for Transfusion Medicine of Republika Srpska. During this period blood was collected from 8153 voluntary donors. It was serologically proved that 40 donors (0.49%) had the weak D antigen. All results where the weak D antigen was determined serologically were confirmed by molecular testing. 23 respondents were proved to have weak D type 3 (0.28%), while 17 had weak D type 1 (0.20%).

Summary/Conclusions: The results from the first molecular testing of our population is in accordance with the results of frequency of weak D antigen in the populations of other European countries, though it did show a small advantage of weak D type 3 over weak D type 1.

P-509

COMPLETE SEQUENCING OF THE RHD BLOOD GROUP GENE USING NEXT GENERATION SEQUENCING: DETERMINATION OF RHD REFERENCE SEQUENCES

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Background: The (Rh) blood group system (004) is the second most important blood group after ABO. Two closely related genes, *RHD* and *RHCE*, encode over 50 different antigens in the Rh system. Recombination, deletion, and point mutations in these genes generate Rh allelic diversity which makes the Rh blood group the most polymorphic blood group system. In the past decade, different DNA microarray-based tests were introduced that enable genotyping of variant blood group specific single nucleotide polymorphisms (SNPs). However, these assays have limitations because they target certain nucleotides or DNA regions through PCR, while novel variants remain unknown. Complete DNA sequencing could be the most relevant technique to thoroughly study blood group variations. Complete blood group genotyping could decrease Rh mistyping and eventually minimise the adverse reactions following a blood transfusion especially for blood transfusion dependent patients.

Aims: This research aims to use Next Generation Sequencing (NGS) to sequence the *RHD* gene to detect *RHD* variants present in the population, which will help expand the knowledge about the underlying molecular mechanism of these variants. This research also focuses on investigating intronic SNPs that could be linked to a specific haplotype that might be used in the future to predict Rh haplotype.

Methods: Genomic DNA samples from blood donors of different phenotypes including 6 R_1R_1, 6 R_2R_2, 7 R_1R_2, 6 R_1r, 6 R_2r, and 6 R_0r were sequenced using the Ion Personal Genome Machine™ (PGM™). All samples were tested for *RHD* zygosity using digital PCR. The *RHD* gene was amplified in 6 overlapping amplicons using *RHD*-specific primers. 200-base pair read sequencing libraries were prepared and then sequenced on the Ion PGM™ using a 316 chip. Data was then mapped to the *hg38* human genome reference sequence and analyzed using the CLC Workbench 9.5.

Results: In one R_2R_2 sample, one exon 9 SNP 25321889 G>C was detected resulting in the amino acid change Gly385Ala which is linked to weak D type 2. Multiple intronic SNPs were detected in all samples in which 15 homozygous SNPs were present in all 37 samples, these may represent SNP variants of the *DAU*0* allele which the hg38 reference sequence encodes. Another 19 SNPs were present in all R_2R_2 and R_2r samples as homozygous SNPs and in R_1R_2 samples as heterozygous SNPs. 14 intronic SNPs were present in all R_1R_1, R_1r and R_0r as homozygous SNPs and heterozygous SNPs in all R_1R_2 samples. 16 heterozygous intronic SNPs were only present in the R_2R_2 weak D type 2 sample. Intronic SNPs are suspected to be linked to a specific haplotype, which could be used in the future to establish an assay to genotype Rh antigens without the need to fully sequence the Rh genes.

Summary/Conclusions: In this research, 37 samples were sequenced on the Ion PGM™ to study *RHD* mutations and assess *RHD* variations present in the population. Further samples are currently being sequenced using identical techniques. Intronic SNPs were used to determine their relation to specific haplotypes. They may represent novel diagnostic approaches to investigate known and novel variants of *RHD* and *RHCE*. The sequencing of multiple hemizygous samples results in the determination of reference *RHD* genes, which we will describe.

P-510

IDENTIFICATION OF WEAK D TYPE 100 IN TWO UNRELATED ITALIAN DONORS

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Background: Among variants of the RhD antigen, Weak D types are characterized by a decreased number of antigenic sites on the erythrocyte membranes. Molecular

bases of these alterations have been studied for many years, so that nowadays more than 100 different alleles are known that lead to Weak D phenotypes.
Aims: One topic of interest in the characterization of Weak D types is the investigation of their territorial distribution. Here we report the identification of Weak D type 100 in two Italian unrelated blood donors; to the best of our knowledge, this is the first time this RhD variant is detected in the Italian population.
Methods: RhD typing in blood donors was carried out by microplate direct agglutination (Galileo-Neo, Immucor), with the use of 2 different anti-D IgM clones [Clone 1 (DVI+): LDM1 + ESD1M; Clone 2 (DVI-): RUM-1, TH28]; on RhD-negative samples, the presence of weak D antigens was evaluated with solid phase technology, by using 2 different anti-D IgG clones [Clone 1: MS26; Clone 2: D415 1E4]. Two donors showed Weak D features in direct agglutination tests (discordant reactivity with the 2 clones, resulted negative and positive, respectively). Based on our diagnostic algorithm for the investigation of RhD serological discrepancies, a further analysis was done both in test tubes with additional anti-D IgM and IgG sera (Bio-Rad, Diagast), and in gel cards with an extended anti-epitopes panel (Extended Partial RhD Typing Set, Bio-Rad). A serologic pattern typical of a weak D variant was confirmed but a classification with conventional methods was not possible. After DNA extraction the 2 donors were genotyped with both the RHD BeadChip kit (Immucor/BioArray Solutions) and SSP-PCR assays (BAGene and Inno-Train); finally, due to molecular ambiguities, the whole *RHD* coding region (10 exons) was subjected to direct sequencing analysis.
Results: SSP-PCR tests consistently resulted in the determination of a DVa variant, while in the RHD BeadChip analyses a homozygous/hemizygous c.787G>A mutation (G263R) was clearly visible, with no additional alterations at any other genetic marker included in the RHD BeadChip panel. Keeping into account the discordancy in the results of molecular assays, and due to the association of c.787G>A mutation to other *RHD* gene variations in several known D variants, the 2 samples were studied by direct sequencing analysis of the whole *RHD* coding region. Apart from c.787G>A, no other mutation was found, thus confirming that there was a unique *RHD* alteration in exon 5 in these donors.
Summary/Conclusions: The c.787G>A mutation introduces a Gly>Arg amino-acidic substitution at residue 263 of the RhD polypeptide. The resulting Weak D phenotype has been recently assigned the definition of "Weak D type 100" by the ISBT committee (RHD*01W.100). This variant belongs to the Eurasiatic cluster, shows mildly weak serologic reactivity and is usually associated to the RhCe phenotype. Being very rare, it has been previously reported exclusively in Japanese and Chinese donors. To the best of our knowledge, this is the first time the presence of Weak D type 100 is reported in a European population.

P-511

A THIRD ABO ALLELE IN A PATIENT WITH TRISOMY 9

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Background: Serological ABO typing in a young female patient resulted in a blood group A phenotype. A weak expression of the B antigen was assumed. No anti-A or anti-B was present in the patient's serum. Phenotypes were established by standard serological methods using tube and/or gel-column agglutination techniques (Bio-Rad). Surprisingly, commercial ABO PCR-SSP by different suppliers (BAG Health Care, Lich, Germany; Innotrain, Kronberg, Germany) detected either an O^1B *or* $O^{1v}B^1$ genotype. No *A* genotype was detectable using this commercial kits. Upon further inquiry the clinicians confirmed a trisomy 9 of the patient.
Aims: The possibility of three *ABO* alleles encoded by chromosome 9 as reason for the inconsistent typing results should be ruled out by DNA sequencing.
Methods: *ABO* sequencing was performed as cycle sequencing of exons 1–7 including flanking intron sequences and the enhancer *CBF/NF-Y* region of the promotor applying the Big Dye Terminator v3.1 chemistry (ABI, Weiterstadt, Germany) followed by electrophoretic separation in an ABI Prism 310 DNA analyzer. Additionally, an A-haplotype specific sequencing of exons 6 and 7 was added in order to separate the *A* allele from the remaining haplotypes. Determined DNA sequences were aligned to published reference sequences.
Results: Generic DNA sequencing could not unequivocally determine the correct *ABO* alleles. Besides an *ABO*O2* allele determined by *106T, 188A, 189T, 220T, 261delG, 646A, 681A, 771T* and *829A* and an indefinite *A* allele, very small *B*-specific peaks were visible in the electropherogram. The enhancer *CBF/NF-Y* region of the *ABO* promotor covered one single and a fourfold repetitive 43 bp motive. The *A*-haplotype specific sequencing of exons 6 and 7 demonstrated an *ABO*A105*, including *467T* in combination with an **O02*, including *261delG* and a small amount of estimated 7% of **B103,* which was determined by additional very low *526G, 703A, 796A, 803C* and *930A* peaks.
Summary/Conclusions: The existence of more than two ABO alleles in a patient with trisomy 9 could only be unambiguously determined by a haplotype specific sequencing approach. An *ABO*A105* allele (not detectable in PCR-SSP) was compatible with the blood group A phenotype and an *ABO*B103* allele in a low quantity was responsible for the assumed weak expression of B in serological typing. These results are consistent with a trisomy 9 mosaic which is characterized by an only partial penetration of the third chromosome within somatic cells.

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TWO NOVEL VARIATIONS IN EXON 7 OF A ALLELE IS RESPONSIBLE FOR A WEAK PHENOTYPE

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Background: The ABO blood group system is highly important in clinical transfusion and transplantation medicine. Lot of weak expression of ABO blood group have been discovered, which not only caused by the single nucleotide polymorphisms (SNP), hybrid formation between the common alleles, mutation in the untranslated region (UTR), but also relate to various mutations changing glycoprotein structure of ABO gene.
Aims: This study aims to investigate the molecular basis of ABO gene in a patient with serologic ABO blood group discrepancy.
Methods: The patient enrolled was from Shanghai Children's Medical Center. Serologic blood group identification, Coombs' test and antibody screening were detected with DG Gel Confirm cards, Neutral cards, Coombs cards by WADiana/8XT Compact Analyzer (from Diagnostic Grifols, S.A). The enhancer, promoter, exon 1~7 and their adjacent intron region of ABO gene were amplified by using polymerase chain reaction (PCR) method, the PCR products were directly or by TA cloning sequenced to identify the gene mutation.
Results: The patient's red blood cells showed two groups in gel and mixed field in tube with monoclonal anti-A, strong agglutination with monoclonal anti-B(++++) and weak agglutination with anti-H (++). The patient's serum showed no agglutination with A1 cell, B cell and O cell. The direct antiglobulin test (DAT), indirect antiglobulin test (IAT) and antibody screening were all negative.
The ABO gene sequencing result showed several variants in exon 6 and exon 7. One heterozygous variation in exon 6 (297A>G) and nine heterozygous variations in exon7 (467C>T, 526C>G, 657C>T, 703G>A, 796C>A, 803G>C, 821A>C, 828C>G 930G>A) of ABO gene were identified in patient compared with the reference sequence of A101 allele. Two of these variations were novel (821A>C and 828C>G). There was no mutation in noncoding region.
Summary/Conclusions: Based on the Blood Group Antigen Gene Mutation Database and Genbank gi734655974, 467C>T was the characteristic of A102 and 297A>G, 526C>G, 657 C>T, 703 G >A, 796 C >A, 803G>C, 930G>A were characteristics of B101. The patient was identified as A102/B101. The reason for A102 weakened expression was 821A>C and 828C>G mutations in exon 7 causing 266Met>Leu and 268Ala>Gly. The location of the two amino acids is very close, which may cause the change of the structure and affect the expression of the antigenic determinant, which causes the weak expression of AB phenotype.

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AN ABO MOLECULAR TYPING ENIGMA

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Background: Molecular methods are increasingly used as complementary approach for blood type determination. A deep understanding of the relationship of genotype and phenotype is a prerequisite for a possible future replacement of serology by molecular typing. Hence, a work-up of atypical observations seems mandatory to assure the feasibility of such future approach.

Aims: We present a case report of a blood donor with an Ael phenotype and discrepant molecular typing results including a prediction of blood type O initial sequencing.
Methods: Serologic blood group determination was initially done on a PK 7300 (Beckman-Coulter) using Bioclone Anti-A (Ortho, clones MH04, 3D3) and confirmed on Bio-Rad ID ABO/RhD card; for reverse testing, in-house cells were used. Adsorption/Elution was tested with Ortho Anti-A and acid elution. RBC Ready Gene ABO (Innotrain), BAGene ABO Type (BAG) and an in-house ABO multiplex PCR were used for ABO typing, sequencing of ABO exons 1–7 were performed from genomic DNA by direct taq cycle sequencing using BigDye-terminators v1.1 in an ABI 310.
Results:: Both by PK 7300 and in ID system, the red cell phenotype was determined as O. Reverse testing revealed only anti-B (4 +), while A1 and A2 cells were negative. On adsorption/elution, the eluate contained an anti-A, hence an Ael phenotype was assumed. On molecular testing, the Innotrain RBC Ready gene ABO kit suggested A01/O01 but could not identify the cause of the diminished A expression. Further analysis by BAGene ABO type variant, in-house multiplex PCR and ABO sequencing seemingly revealed the genotype O01/O01 but could neither elucidate the basis for the Ael phenotype nor explain the predicted A01 allele in the RBC ready gene kit. The results were classified as contradictory and an independent sample was obtained. Finally, sequencing of this sample revealed faint double bands starting just after the 1 bp deletion typical for O01 suggesting that the puzzling genotype was due to a chimeric DNA consisting mostly of genotype O01 with a faint admixture of A01 (a further sequencing of the A allele was not attempted).
Summary/Conclusions: Chimerism in healthy blood donors may pose diagnostic challenges. The serologic phenotype usually suggests a weakened antigen expression. The molecular analysis may be puzzling: Conventional methods are usually aimed to detect mutations causing weakened antigen expression, which are unlikely to be present in such cases. Molecular methods may differ in their sensitivity to admixtures and may thus yield a combination of seemingly contradicting results. Even Sanger sequencing may miss faint admixtures if the analysis is not targeted to the detection of such admixtures. Advanced methods like Next Generation Sequencing or digital PCR may be helpful but even with those methods the challenge of discriminating a faint admixture from background remains.

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MOLECULAR BIOLOGY IN RESOLVING DISCREPANCIES FOR DETERMINING THE ABO BLOOD GROUP

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Background: The possibility of using methods of molecular biology has expanded both the genetic knowledge of blood groups, by overcoming the classic limitations of the hemagglutination tests, both gave the possibility to resolve the discrepancies that there are between blood group direct and indirect, which are frequent in daily laboratory practice. In the transfusion service of Aversa, the whole donor blood are determined through by automated systems in micro-column, and in case of discrepancy, the samples are analyzed through various techniques including those of molecular biology.
Aims: The purpose of the study was to verify how the techniques used have been productive for the correct reporting of the blood groups
Methods: From 2009 to 2016 have been typed more than 90.000 blood donors. The first blood group determination was performed through Ortho Autovue Innova System that uses the technique in micro column, and the discrepancies found were analyzed using the following protocol:

- preparation of the direct and indirect Coombs test (to rule out any interference by autoantibodies and alloantibodies)
- determination of direct and indirect blood group with method in microplates (Galileo Neo Immucor)
- evaluation of the agglutinine at 4°C and 37°C
- in the case of difficult resolution, SSP-PCR(Single specific primer-polymerase chain reaction) is performed

Results: A total of 90,000 typifications, were recorded 434 ABO discrepancies, of these:

1. 352 were solved with determination of agglutinins at 4°C.
2. 48 caused by the presence of autoantibodies
3. 32 caused by the presence of alloantibodies
4. 2 cases solved by molecular biology tests because they were antigenic variants, and in the specific "Ael" that appears to be a variant of "A"

Summary/Conclusions: Thanks to the protocol prepared we were able to identify two donors with Zero group and which showed positive reaction to anti B and negative reaction to anti A, A1, AB and H. With the support of molecular biology have typed the genotype of donors that is found to be Ael, which is a subgroup of the ABO system that does not present surface antigens. Blood products obtained from donations of blood were discarded for patient protection, in the donor's report, it is specified that in case of possible transfusion, he must be regarded as a patient with zero group

P-515

BW14 SUBTYPE IDENTIFICATION WITH ITS MOLECULAR BASIS

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Introduction: ABO is the most important blood group system in transfusion medicine. ABO typing discrepancy was occasionally seen in clinic. Here, we reported an analysis of all the exons of the ABO gene of one AML case with forward typing and reverse typing discrepancy referred to our lab to identify the ABO molecular genetic basis of this patient.
Methods: On the basis of standard serological assay, ABO subtype and haplotype analysis were performed by PCR amplification of seven exons and their adjacent introns of the *ABO* gene and by TA clone sequencing.
Results: ABO forward typing showed B type, and reverse typing demonstrated an extremely weak anti-B in routine gel test which indicated ABO forward and reverse typing discrepancy. Absorption-elution test confirmed that there was no A antigen on the surface of patient's RBCs.The ABO gene sequencing showed a G>A exchange at position 523 in exon 7 which resulted in a Val to Met substitution at codon 175. The ABO gene amplified products and clone sequencing analysis showed an ABO* Bw14/O01 heterozygote genotype for this patient.
Conclusion: The method of molecular biology is necessary for identifying ambiguous blood groups. A 523G>A substitution of the ABO gene resulting in a Bw14 subtype was one of the molecular basis of its weak B phenotype.

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PRECISE ABO GENOTYPING TO DISTINGUISH INHERITED OR ACQUIRED ABO VARIANTS

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Background: The genotyping of ABO locus is useful to solve some ABO grouping discrepancies of serological method. Most genotyping methods focus on the exon 6 and 7 of ABO gene to predict ABO phenotypes, but some genotyping results cannot explain the serological discrepancies. There should be developed a more effective strategy to identify ABO genotypes accurately.
Aims: In this study, we developed a precise genotyping strategy to distinguish inherited or acquired ABO variants.
Methods: The ABO gene was analyzed on all variant samples which screened out by serological tests. The analysis methods were selected step by step according to the analysis strategy. Briefly, the exon 6 to 7 and flanking splice site sequences were detected firstly by direct DNA sequencing method. The ABO genotypes were assigned directly if the nucleotide sequences were fully matched to any known subgroup alleles. Otherwise, exon 1 to 5 and flanking sequences were analyzed further. Similarly, analysis of intron 2 to 6, expression regulatory element in intron 1, promoter and enhancer in 5′-untranslation regions (5′-UTR) was performed step by step according to the gene character of samples. A haplotype analysis was also performed using cloning sequencing if novel mutations or ambiguous genotypes were found. The samples were categorized as inherited ABO variants if mutations were detected in analysis regions; otherwise, acquired ABO variants were categorized. The work was supported by Science Research Foundation of MOH, China (WKJ-ZJ-1510) and Zhejiang High-Level Innovative Health Talents.
Results: Total of 188 serological variants were analyzed according to our genotyping strategy. 122 samples (64.9%) were identified as inherited ABO variants, while 66 samples (35.1%) were suspected to acquired ABO variants. In inherited ABO variants, although most samples (n = 92) had been detected crucial nucleotide mutations in the exon 6 or 7, there were still 24.6% samples (n = 30) which were found mutations outside the exon 6 and 7. In these variants, 24 samples were found mutations

in the exon 1, and 5 samples were found mutations in expression regulatory element of the intron 1, and 1 sample was found higher methylation in the CpG island of promoter region. In acquired ABO variants (n = 66), 41 samples were found weak antigens expression as a result of hematologic diseases (n = 33) or absence of any obvious disease (n = 8). Moreover, 19 samples were identified as weak ABO antibodies or presence of unexpected antibodies in serum due to the physiological or pathological factors. 6 samples were found to have acquired B antigen or polyagglutination phenomenon due to microbial infections.
Summary/Conclusions: The improved ABO genotyping strategy, which sequentially analyzed exons, introns and regulatory regions, can distinguish the inherited and acquired ABO variants. It is more precise than traditional methods focused only on exon 6 and 7 of ABO gene.

P-517
POLYMORPHISMS OF FUCOSYLTRANSFERASE 2 (FUT2) GENE IN BLOOD DONORS OF TEHRAN, IRAN

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Background: Fucosyltransferase 2 (*FUT2*) gene encodes the α(1,2) fucosyltransferase enzyme that regulates the expression of ABH antigens in body fluids. *FUT2* gene is highly polymorphic and many ethnic-specific alleles have been identified.
Aims: The aim of the present study is to determine the frequency of common *FUT2* alleles in blood donors of Tehran, Iran.
Methods: A total of 246 blood samples were analyzed from blood donors of Tehran. The genotype of se^{428}, Se^{357} and $Se^{357\ 480}$ alleles of *FUT2* gene were determined by polymerase chain reaction with sequence-specific primers (PCR-SSP). Finally a multiplex PCR was designed to detect these three alleles in a single reaction.
Results: The frequency of se^{428}, Se^{357} and $Se^{357,\ 480}$ alleles were 0.443, 0.321 and 0.128, respectively in our population.
Summary/Conclusions: The frequency of se^{428} and Se^{357} alleles were approximately the same as in Caucasian and African populations but $Se^{357,\ 480}$ was found in a higher frequency compared to those populations.

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MUTATIONAL ANALYSIS OF BOMBAY PHENOTYPE IN IRANIAN PEOPLE: IDENTIFICATION OF A NOVEL FUT1 ALLELE

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Background: Bombay phenotype is characterized by lack of ABH antigens on RBCs and in body secretions as a result of mutations in fucosyltransferase 1 (*FUT1*) and fucosyltransferase 2 (*FUT2*) genes.
Aims: The aim of this study was a mutational analysis in Iranian people with Bombay phenotype.
Methods: ABO genotyping and serological analyses including adsorption-elution tests were performed in five unrelated Bombay individuals. The coding regions of *FUT1* and *FUT2* genes were amplified and sequenced directly or after cloning into suitable vector.
Results: A novel (G1051T; G351C) and four reported *FUT1* alleles were revealed. Molecular analysis of *FUT2* gene confirmed nonsecretor status in all individuals.
Summary/Conclusions: A new nonfunctional *FUT1* allele was detected. This and our previous findings suggest the diversity and population specificity of *FUT1* alleles.

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A FUT1*C.787INSA MUTATION INDUCES A PREMATURE STOP AND THE BOMBAY (H-) PHENOTYPE

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Background: Mutations of the *FUT1* gene can abolish the alpha 1,2 fucosyltransferase activity and induce the A-, B-, H- phenotype on red blood cells. In combination with further mutations of the *FUT2* gene, which regulates the secretor function, the synthesis of substance H can be completely inhibited on red blood cells and within plasma as well, which leads to the Bombay (O_h) phenotype. This very rare phenotype has been especially described in individuals from Southern Asia, but also cases from Europe have been reported. We describe the Bombay phenotype in a woman of Turkish origin, living in Southern Germany whose blood had been submitted for testing by a fertility centre because of abnormal immunohaematological results.
Our serological findings were typical for the Bombay blood group, with a strong positive antibody test, negative DAT and an A, B and H negative phenotype.
Aims: The molecular basis of the Bombay (O_h) phenotype of the patient should be elucidated by DNA sequencing.
Methods: CDS exon 1 of both *FUT1* and *FUT2* genes including short flanking sequences of the untranslated regions were amplified in a gene specific manner by use of published primer sequences. Cycle sequencing was performed with the Big Dye Terminator v3.1 chemistry (ABI, Weiterstadt, Germany) followed by electrophoretic separation in an ABI Prism 310 DNA analyser. Determined DNA sequences were aligned to published reference sequences.
Results: DNA sequencing demonstrated a *FUT1*c.787insA* mutation which induces a frame shift and a premature stop at codon 269 instead of codon 365. Abolishing the function of the fucosyltransferase no H-substance could be synthesized on the patients' red blood cells. A number of already known mutations of the *FUT2* gene induced the *sese* genotype and thus prevented secretion of substance H into the plasma: *171A>G* (silent), *216C>T* (silent), *428G>A* (Trp143Stop), *739G>A*, *960A>G*. The mutations of both genes induced the O_h phenotype. The new *FUT1*787insA* mutation was submitted to GenBank under the accession number KY593920. This is our 5th O_h case within 3 years in patients or donors of non-Middle European origin.
Summary/Conclusions: The new *FUT1*c.787insA* mutation in combination with a *FUT2*c.428G>A* mutation induces the Bombay (O_h) phenotype in a patient of Turkish origin.

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WEAK B PHENOTYPE CAUSED BY A NOVEL PARA-BOMBAY MUTATION

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Background: The expression of ABO antigens is dependent on H precursor chain synthesis. H-deficient phenotypes have been described on numerous genetic backgrounds with mutations causing loss of α2-fucosyltransferase activity in haematopoietic (*FUT1*) and secretory (*FUT2*) tissues. No A, B or H antigens are expressed when both transferases are nonfunctional (i.e. the O_h or Bombay phenotype), however weak expression of A and/or B may be detected when the mutations are only in *FUT1* and the α2-FucT2 transferase encoded by *FUT2* is active (i.e. para-Bombay phenotype). In these individuals, soluble A and/or B antigens are adsorbed from plasma onto the red blood cells (RBCs).
Aims: A discrepancy in the ABO grouping of a healthy pregnant woman (Caucasian, age 33) prompted the investigation of the phenotype, genotype and flow-cytometry profile.
Methods: Routine ABO grouping was performed by the column agglutination (Bio-Rad and Grifols), microplate test (Bio-Rad) and tube test (Immucor). H, I and i expression was tested in neutral cards (Bio-Rad) using anti-H lectin and anti-I and anti-i polyclonal sera. *ABO* and *FUT2* genotyping was performed by routine PCR-ASP and PCR-RFLP. The coding region of *FUT1* was investigated by Sanger sequencing and analyzed using CodonCode Aligner software. RBCs were tested monoclonal anti-A, anti-B and anti-H by flow cytometry.

Results: The patient's RBCs initially typed as group O but she lacked anti-B in her plasma. No B antigen was detected in the Bio-Rad ABO cards however weak B expression was observed in the ABO DG Gel cards and in the tube test. In reverse grouping, no anti-B agglutinin was detected in the two systems of column agglutination. Her RBCs typed H with anti-H lectin, and Le(a b+) indicating secretor status, and unusually, showed strong reactivity with both anti-I and anti-i. The *ABO* genotype of the proband was *ABO*B.01/O.01.01,* consistent with normal group B. *FUT2* analysis revealed homozygosity for *FUT2*01*, consistent with secretor status. However, sequence analysis of *FUT1* revealed homozygosity for a new mutation – duplication of 7 nucleotides, c.13_19dup, which introduces a frameshift and an early stop codon (p.Arg7Glnfs*63). Flow cytometric analysis confirmed the absence of H antigen on the proband's RBCs but weakly expressed B antigen was clearly demonstrable.

Summary/Conclusions: We report a para-Bombay phenotype (B_h phenotype, H-deficient, secretor) in a young woman in whom homozygosity for a novel *FUT1* mutation was identified. This reading frame altering mutation most likely does not result in a translated fucosyltransferase. The weak B expression [HA1] probably results from the absorption of soluble B antigen from the plasma. The unusual strong i antigen expression will be further investigated.

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MULTIPLE MISCARRIAGES IN TWO SISTERS OF THAI ORIGIN WITH THE PK PHENOTYPE CAUSED BY A NOVEL NONSENSE MUTATION AT THE B3GALNT1 LOCUS

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Background: Globoside, the most abundant neutral glycosphingolipid in the red blood cell (RBC) membrane, is also known as the P antigen in the GLOB blood group system. The antigen is expressed in several other tissues throughout the body, including the placenta. The very rare P/PX2-negative P^k phenotype arises from the absence of a functional P synthase due to inactivating mutations in the 3-β-*N*-acetylgalactosaminyltransferase gene (*B3GALNT1*). The common P1 + (P_1) and P1– (P_2) phenotypes depend on a single polymorphic nucleotide position in a regulatory region of the 4-α-galactosyltransferase gene (*A4GALT*), which encodes the P^k synthase. Thus, P/PX2-negative individuals can have either the P_1^k or P_2^k phenotypes. In analogy with the ABO system, their sera contain naturally-occurring antibodies directed against the antigens lacking. The most potent specificity is anti-P that may cause serious intravascular haemolytic transfusion reactions. Furthermore, women with P_1^k/P_2^k phenotypes have a higher incidence of early spontaneous abortions due to cytotoxic attack by anti-P on the globoside-rich placental tissue.

Aims: To determine the genetic background resulting in the rare P^k phenotype in two sisters of Thai origin with multiple spontaneous abortions and naturally-occurring anti-P.

Methods: Serological and genomic analysis was performed on blood samples from the two sisters, following informed consent. The presence of P, P1 and P^k antigens on RBCs and antibody specificities in plasma were determined by standard haemagglutination techniques and flow cytometry. DNA was isolated and the coding region of *B3GALNT1* was amplified using standard PCR and subsequently analysed by Sanger sequencing. Genotyping for a P_1/P_2-differentiating *A4GALT* polymorphism was performed with a custom-designed allelic discrimination (AD) assay.

Results: Medical records showed that the two sisters had suffered 11 and 8 miscarriages, 18 of which occurred at 8–12 weeks of pregnancy. Strongly reactive anti-P and weak anti-PX2 was identified in their plasmas. Flow cytometry showed both samples to be P PX2 P^k+(strong). RBCs from one sister typed P1+, the other P1 . Serological results were corroborated by the P_1/P_2-differentiating *A4GALT* assay, which confirmed heterozygosity for P^1/P^2 in the P1 + sample and homozygosity for P^2 in the P1 sample. Sequencing revealed homozygosity for a novel nonsense mutation, c.420T>G, in the *B3GALNT1* coding region in both sisters. This substitution introduces a premature stop codon, p.Tyr140Ter, leading to a severely truncated gene product. The resulting protein is predicted to lack all enzymatic activity and thereby explains the rare P^k phenotype and the corresponding antibodies. Since inactivating mutations in *B3GALNT1* and other null blood group phenotypes have been shown to be regional and very little is known about *B3GALNT1* polymorphism among the Thai, an allele-specific primer (ASP) PCR detecting the novel mutation was designed for screening of a cohort of Thai blood donors.

Summary/Conclusions: We describe a previously unreported mutation (c.420 T>G) in *B3GALNT1*, which had resulted in the P^k phenotype in two sisters with an unusually sad history of multiple miscarriages and no live children due to their naturally-occurring anti-P. As a result of this study, yet another null allele of the GLOB system could be added to the twelve already acknowledged by ISBT.

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AN EXONIC MISSENSE MUTATION C.28G>A DECREASES ABUNDANCE OF MRNA FOR ABO GENE AND IS ASSOCIATED WITH WEAK B BLOOD TYPE

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Background: The amino acid substitutions caused by ABO subgroups associated mutations are usually predicted to impact glycosyltransferase's function or its biosynthesis.

Aims: Here we reported an ABO exonic missense mutation that affects B antigen expression by decreasing stability of mRNA transcripts of *ABO* gene rather than the amino acid change.

Methods: Serological studies including total plasma GTB transfer capacity were performed. *ABO* gene was analyzed by sequencing. *B* cDNA with c.28G>A (p.G10R) mutation was expressed in HeLa cells and total GTB transfer capacity in cell supernatant was measured. Flow cytometry was performed on these HeLa cells post-transfection, and agglutination of Hela-B_{end} cells was also examined with anti-B antibody. The mRNA of *ABO* gene was analyzed by direct sequencing and real time RT-PCR.

Results: While plasma total GTB transfer capacity was undetectable in this B_{end} individual with c.28G>A mutation, B antigen presented on 19% RBCs and MFI of the B antigen on RBC was 14% of normal B RBCs. There was no significant difference of total GTB transfer capacity in cell supernatant and B antigen expression on cell surfaces between HeLa-B_{end} and HeLa-B cells. *B* mRNA transcripts with different size were failed to find in this individual. The transcripts of the *ABO* gene containing 28G>A in peripheral whole blood was significantly reduced.

Summary/Conclusions: *ABO* exonic missense mutation c.28G>A may cause weak B subgroup by decreasing abundance of mRNA for ABO gene rather than changing primary structure of protein.

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Abstract has been withdrawn.

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P1PK BLOOD GROUP SYSTEM: HOW SINGLE NUCLEOTIDE POLYMORPHISMS AFFECT THE NUMBER OF SURFACE ANTIGENS

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Background: P1PK blood group system consists of 3 glycosphingolipid antigens: P^k, P1 and NOR. Presence or absence of P1 antigen determines P_1 and P_2 phenotype, respectively. Despite recent progress, the molecular background of P_1/P_2 blood group polymorphism still remains elusive. All P1PK antigens can be synthesized by α1,4-galactosyltransferase, which is encoded by *A4GALT* gene; it was shown that

rs8138197 (Thuresson, Blood, 2011) and rs2143918 and rs5753148 (Lai, Transfusion, 2014) found downstream of Exon 1 are associated with P_1/P_2 phenotype. In addition, c.631C>G missense mutation causes broadening of the enzyme's specificity, which in addition to P^k and P1 becomes able to synthesize NOR antigen.

Aims: To evaluate the relationship between described SNPs and the number of P1PK antigens on RBCs, we determined SNPs (rs8138197, rs2143918, rs5753148) in 85 individuals of known phenotypes (P_1, P_2, P_1NOR), evaluated *A4GALT* transcript level and anti-P^k/P1/NOR antibody binding capacity.

Methods: DNA and RNA was isolated from whole blood. Real-Time PCR of *A4GALT* was performed using actin as the endogenous control. Anti-P^k/P1 antibody binding capacity was evaluated by flow cytometry using Qifikit quantitative assay. Antibodies: mouse monoclonal anti-P1 (CE Immunodiagnostica, clone 650; recognizes P^k and P1), human monoclonal anti-P1 (Immucor, clone P3NIL100; recognizes P1 only), mouse monoclonal anti-NOR (clone nor118; recognizes NOR1 and NOR2). Data are presented in the form of scatter plots.

Results: We found that rs5753148 (Lai, Transfusion, 2014) reveals the strongest association with anti-P^k/P1 antibody binding capacity and *A4GALT* transcript level. The number of P^k/P1 antigens in NOR-positive individuals was significantly lower than in NOR-negative individuals.

Summary/Conclusions: While RBCs of P^2P^2 genotype bound neither mouse anti-P1 monoclonal antibody nor human anti-P1, RBCs of P^1P^1 genotype bound statistically more antibodies of each specificity than P^1P^2 RBCs. However, scatter plots of genotype versus antibody binding capacity revealed a skewed distribution within each genotype group with a substantial overlap between P^1P^1 and P^1P^2, suggesting that P_1/P_2 phenotype cannot be universally predicted based on the known SNPs and that there are confounding factors that affect the phenotype, which are yet to be discovered. In addition, presence of NOR-causing mutation in α1,4-galactosyltransferase results in 29% decrease of P1 synthase activity.

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RESOLUTION OF LUTHERAN TYPING DISCREPANCIES DUE TO AN IN(LU) PHENOTYPE

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Background: The Lu(a-b-) phenotype arises from 3 distinct genetic backgrounds: (i) homozygosity for inactivating mutations in the *BCAM (LU)* gene resulting in rare Lu_{null} phenotype, no antigens of LU system can be detected; (ii) heterozygosity for mutation in *KLF1* gene resulting in In(Lu) phenotype, characterized by a reduced expression of Lu, AnWj and other antigens outside of the LU system; (iii) variations in the X-chromosome-borne *GATA1* gene resulting in Lu(a-b-) phenotype through inactivation of the erythroid transcription factor GATA-1. The *BCAM* is located on the chromosome 19 and comprises 15 exons, whereas *KLF1* is also located on the chromosome 19 and comprises 3 exons. We describe several Lutheran discrepancies between serological and molecular typing due to an In(Lu) phenotype.

Aims: We investigated the discordance observed in 12 Caucasian and 1 Filipino blood donors phenotyped as Lu(a-b-) but genotyped as *LU*A/LU*B* and *LU*B/LU*B* in 1 and 12 donors respectively.

Methods: Lu phenotyping was performed using Neo Galileo instrument (Immucor, USA) and tube methods with commercial polyclonal anti-Lu^a and anti-Lu^b reagents (Immucor Germany; CE-Immunodiagnostika Germany; Bio-Rad Switzerland). Genomic DNA was isolated from whole blood using QIAamp DNA Blood Kit (QIAGEN, Germany) and the *LU* genotype was done by HEA BeadChip™ kit (BioArray Solution, USA) and PCR-SSP kit (RBC-Ready Gene Rare ID, Inno-Train, Germany). Since the phenotype and genotype were discordant, the samples were further characterized by sequencing (BigDye Terminator v3 on the AB3730 DNA Analyzer, Thermo Fisher; Geneious R10.0.9).

Results: In according to our policy, 13 donors with Lu(a-b-) phenotype were genotyped: 12 had a Lu(a-b+) and 1 Lu(a+b+) predicted phenotype by HEA and RARE ID kits. In addition, the RBCs were non-reactive with multiple anti-Lu^a and anti-Lu^b antisera. Sequence analysis of the *LU* and *KLF1* genes showed that 12 individuals were Lu^b homozygous and 1 Lu^b heterozygous but all were also heterozygous for mutations in the *KLF1* resulting in In(Lu) phenotype. Full-gene *KLF1* sequencing found a missense mutation at *c.304T>C* (p.Ser102Pro) in exon 2 in 4 donors and a nonsense mutation at *c.809C>A* (p.Ser270Ter) in exon 2 in 2 donors, both previously described. In the remaining donors, we found novel alleles due to single nucleotide substitutions, deletions or novel combinations: nonsense mutation at *c.313C>T* (p.Gln105Ter) in exon 2 in 1 donor; one nucleotide deletion at *c.512delC* (p.Pro171Argfs*65) in exon 2 in 1 donor; a missense mutation at *c.304T>C* in combination with one nucleotide deletion at *c.569delC* (p.Pro190Leufs*45) in exon 2 in 2 donors; a missense mutation at *c.311C>T* (p.Ala104Val) in exon 2 in combination with two nucleotide deletion at *c.1000_1001delAC* (p.Thr334Glyfs*351) in exon 3 in 1 donor; two nucleotide substitutions at positions *c.304T>C* and *c.886C>G* (p.Leu296Val) in exon 2 in 1 donor. Finally, we found in 1 donor a novel missense mutation *c.1070A>G* (p.His357Arg) in exon 3 of *KLF1* gene in addition to a known missense mutation at *c.292C>T* in exon 3 of the *BCAM* gene.

Summary/Conclusions: The application of DNA methods helped us resolve apparent discrepant results between phenotype and genotype due to *KLF1* alleles responsible for an In(Lu) phenotype.

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A NOVEL KLF1 ALLELE LEADING TO AN IN(LU) PHENOTYPE

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Background: The dominant Lu(a-b-) phenotype, the so-called In(Lu) phenotype, is caused by mutations in the Krüppel-like factor gene *KLF1* on chromosome 19. The protein KLF1 is an erythroid-specific transcription factor crucial for erythropoiesis, the developmental switch from fetal (HbF) to adult (HbA) globin and the expression of beta globin. Additionally to the reduced expression of the Lutheran antigens observed for the In(Lu) phenotype, other antigens such as P1, In^b and AnWj are also suppressed. Several alleles, most as a result of single nucleotide polymorphisms (SNPs), have been reported. Usually these alleles are present in a heterozygous state. Expression of the Lutheran antigens depends on the level of functional KLF1 activity. It was long believed that homozygosity or compound heterozygosity for non-functional *KLF1* alleles is not compatible with life, but one case of compound heterozygosity was reported leading to severe nonspherocytic hemolytic anemia and kernicterus.

Aims: The sample of a donor of Caucasian origin was chosen for genotyping of the 22 most common blood group alleles, including the SNPs for *LU*01* and *LU*02*. An electronic cross-match with the previously determined phenotype revealed a discrepancy for the Lu(b) antigen. The sample was further characterized in our reference lab.

Methods: Phenotyping on ID/IAT-cards (Bio-Rad) was done using anti-Lu(a) and anti-Lu(b) polyclonal antibodies (in-house). The adsorption-elution analysis was performed using a polyclonal anti-Lu(b) antibody. For *LU* genotyping an in-house sequence specific primer (SSP)-PCR method was applied. The sample was further characterized by exon sequencing including flanking intronic regions of the *LU* and *KLF1* gene using published primers for amplification and sequencing.

Results: Repeated standard phenotyping of the sample confirmed the Lu(a) and Lu (b) negativity. The SSP-PCR genotyping method was positive for nucleotide (nt.) 230G (*LU*02*) and the presence of nt.230A (*LU*01*) could be excluded. Sequencing of *LU* confirmed the *LU*02/LU*02* genotype and no mutation was observed in the coding region of the *LU* gene. Sequencing of *KLF1* revealed the mutation c.*330delC* in a heterozygous state. This deletion leads to a frameshift with an aberrant protein sequence starting from amino acid 111 (p.Glu111Argfs*126). To the best of our knowledge the variant *KLF1*330delC* has not been reported previously. The adsorption-elution test with a polyclonal anti-Lu(b) gave a negative result, which is in accordance with the In(Lu) phenotype.

Summary/Conclusions: Here we present a donor of Caucasian origin with an In(Lu) phenotype, resulting from a new *KLF1* null allele, *KLF1*330delC* in heterozygous state (accession number LT796704). Red blood cell concentrates from this donor could be used for transfusion of patients with antibodies against high frequency Lutheran antigens.

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A NOVEL JK*02 SILENT ALLELE CAUSED BY A NUCLEOTIDE INSERTION MECHANISM AND RESPONSIBLE FOR A JK NULL PHENOTYPE IN A PORTUGUESE PATIENT

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Background: The Kidd blood group system consists of three antigens Jk^a, Jk^b and Jk3. Jk^a and Jk^b are polymorphic in all populations. The molecular basis of the Jk^a/Jk^b polymorphism corresponds to a SNP in the *JK* (*SLC14A1* or *HUT11A*) gene in exon 9, c.838A>G (p.Asn280Asp). Jk^a/Jk^b is associated with a synonymous mutation in exon 7, c.588A>G (rs2298718). Jk3 is a high-prevalence antigen, absent in the rare Jk(a-b-) phenotype, also known as Jk_{null}. The Jk(a-b-) type is usually inherited recessively and mainly encountered in Polynesia, Thailand, Taiwan and Finland. Several molecular backgrounds have been reported for Jk_{null}, either on a *JK*01* or *JK*02* allele basis (homozygous or compound heterozygous inactivating mutations). According to the ISBT, 10 silent *JK*01* and 14 silent *JK*02* alleles have been reported, caused by nonsense or false-sense mutations, splice site mutations and deletions causing frameshift; no nucleotide insertion has been described to date.
Aims: We describe here the serological and molecular investigation of a Jk(a-b-) Portuguese patient, with anti-Jk3.
Methods: Antibody screening and identification were performed on native (gel-test, Bio-Rad) and ficin-treated (Panocell-10, Immucor) RBCs. IgG subclass was investigated by a DAT IgG1/IgG3 gel-test. Genotyping was performed with the KKD-Type device (BAG Health Care).
Results: The proband is a group O, R_1R_1, K+, 71 years old female patient, without any known consanguineous genetic background. She had four uneventful pregnancies. In 2004, she was transfused with one RBC unit during an orthopedic surgery; the antibody screening was negative. In 2017, the antibody screening was positive prior to a knee pre-surgical testing (negative autocontrols). The extended phenotype was K+k+, Kp(a-b+), Fy(a+b+), Jk(a-b-). The antibody was confirmed to be anti-Jk3 by using several Jk(a-b-) RBCs. Alloantibodies of common specificities were ruled out. This anti-Jk3 (titer 32) showed a predominant IgG3 subclass. The genotyping results showed no signal for the *JK*01* allele and an extremely weak signal for *JK*02*. Genomic DNA sequencing revealed the presence of an insertion of one nucleotide in exon 9 at position 830 (c.830insC), at homozygous state, responsible for a frameshift and premature stop codon (p.Pro279Ter). The c.588A>G change (rs2298718), associated with a *JK*02* allele, was also found at homozygous state.
Summary/Conclusions: We characterized a novel silent *JK*02* allele, *JK*02 (588G,830insC).* This is the first silent *JK* allele reported to date which involves a nucleotide insertion mechanism. Interestingly, this insertion at position 830 is very close to the Jk^a/Jk^b polymorphism site at position 838. As a result, this may affect the binding of an amplification primer and this is probably why the KKD-Type genotyping device demonstrated an extremely weak signal for the *JK*02* allele. It would be interesting in the future to check whether other genotyping devices are subject to this "allele dropout" mechanism. Of note, allele dropout in this case was consistent with a true Jk(a-b-) phenotype, while a predicted Jk(a-b+) type could be expected in such a molecular background. We suggest this new allele to be named *JK*02N.15*, subject to the agreement of the ISBT Working Party on Red Cell Immunogenetics and Blood Group Terminology.

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IDENTIFICATION IN A CAUCASIAN DONOR OF A NEW JK*B VARIANT ENCODING A NULL PHENOTYPE

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Background: Kidd is one of the simplest blood group systems, consisting of two antithetical antigens (Jk^a, Jk^b) and one antigen of high prevalence (Jk3). The Jk^a/Jk^b polymorphism results from c.838G>A in exon 9, encoding Asp280Asn on the fourth extracellular loop of the Kidd glycoprotein. A variety of mutations are responsible for *JK* silent alleles in several ethnic groups.
Aims: Blood samples from a Caucasian male donor, selected for extended blood group genotyping, were investigated to resolve a discrepancy between the Jk(a+b+) predicted phenotype and the Jk(a+b-) phenotype detected by serology.
Methods: Genomic DNA isolated from buffy coat was genotyped using BLOODchip ID CORE XT™ (Progenika-Grifols, Spain); raw data were analysed with BIDS software (Progenika-Grifols, Spain).
Confirmatory genotyping was performed by PCR-SSP (RBC-Ready Gene KKD, Innotrain, Germany). Serology was performed by the antiglobulin test with human polyclonal anti-Jka and anti-Jkb antisera by the tube, microplate and microcolumn methods (ID-Card, Diamed, Switzerland; Immucor, Germany) and according to manufacturers' instructions. Weak expression of the Jkb antigen was investigated by the adsorption/elution method using Gamma Elu-Kit II (Immucor, Germany).Genomic DNA extracted from buffy coat was PCR amplified with JK-specific primers and the product extended by the Sanger dideoxy method.
Results: Genotyping on ID CORE XT identified a *JK:1,JK:2* genotype for a Jk(a+b+) predicted phenotype. Routine serological typing on the original sample and confirmatory typing on a second sample failed to detect the presence of Jkb antigen. To investigate weak Jkb antigen expression an adsorption/elution test was performed, with negative results. The *JK:1,JK:2* genotype was confirmed on a second sample and also by PCR-SSP. The discrepancy was solved by sequencing, which identified a *JK*A(588), JK*B(157del10)* genotype. *JK*A(588)* is a relatively common allele which encodes a Jka+ phenotype. The variant *JK*B* allele is characterized by a 10-base pair deletion in exon 5, which causes a frameshift and introduces a premature stop codon for a 76-residue truncated protein, providing an explanation for the Jkb null phenotype. The PCR-SSP and ID CORE XT molecular probes do not interrogate *JK* positions c.157–166, which explains the erroneous phenotype prediction.
Summary/Conclusions: We report serological and genetic evidence for a Jk(b-) phenotype resulting from the novel *JK*B(157del10)* molecular background. These findings further add to the genetic complexity of the Kidd blood group system.

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ALTERED JK*A AND JK*B ALLELES ASSOCIATED WITH REDUCED EXPRESSION OF KIDD ANTIGENS

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Background: The Kidd blood group system consists of 3 antigens which are carried on a urea transporter protein encoded by *JK* gene. Several silent *JK* alleles accounting for Jk null phenotype, and mutant *JK*A* and *JK*B* alleles accounting for weak Jk^a and weak Jk^b phenotypes, have been reported.
Aims: We report here novel *JK*A* and *JK*B* alleles detected in Japanese individuals with weak Jk^a and weak Jk^b, respectively.
Methods: Red cells of healthy donors were screened using a monoclonal anti-Jk3 (HIRO-294) by an automated blood grouping system PK7300. Jk^a and Jk^b were typed by standard serology. Genomic DNA was extracted from the blood samples and *JK* gene was analyzed by PCR and sequencing. Coding region of the *JK* gene was amplified by RT-PCR using reticulocyte mRNA and cloned into the plasmid vector (pENTR/SD/D-TOPO, Invitrogen) and mutations were induced using a site-directed mutagenesis kid (Promega). The obtained *JK* genes were subcloned into the expression vector (pIRES2-EGFP, Clontech) then transferred to CHO cells. Expression of Jk^a and Jk^b antigens on the cells were analyzed by flow cytometry (FCM) and relative fluorescence intensity (RFI) was calculated.
Results: We examined 3 blood samples and serological results indicated that these phenotypes were Jk($a+^wb-$), Jk($a+^wb+$), and Jk($a-b+^w$). Sequence analysis revealed that the individual-1 with Jk($a+^wb-$) was homozygous for *JK*A* allele having a novel c.356C>T (p.Ser119Phe) mutation and the individual-2 with Jk($a+^wb+$) had the same mutant *JK*A* allele with common *JK*B*. The individual-3 with Jk($a-b+^w$) had a silent *JK* allele (*JK*02N.09*) with c.191G>A (p.Arg64Gln) mutation and a novel *JK*B* allele with c.284C>T (p.Thr95Ile) mutation. Expression study using the CHO cells revealed that the *JK*A356T*-induced cell was 27% of Jk3 antigen and 22% of Jk^a antigen as compared with the common *JK*A*-induced CHO cells. Similarly, the *JK*B284T*-induced cell expressed 45% of Jk3 antigen and 30% of Jk^b antigen as compared with the CHO cells induced common *JK*B*.
Summary/Conclusions: We identified two novel alleles, *JK*A* with c.356C>T (p.Ser119Phe) and *JK*B* with c.284C>T (p.Thr95Ile), accounting for weak Jk^a (and Jk3) and weak Jk^b (and Jk3), respectively. Both mutations were responsible for reduced expression of Kidd glycoprotein.

P-530

IDENTIFICATION OF A NEW FY*02 NULL ALLELE IN A CAUCASIAN BLOOD DONOR WITH A FY(A-B-) PHENOTYPE

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Background: The Duffy blood group system includes two antithetical antigens encoded by codominant alleles *FY*A* (*FY*01*) and *FY*B (FY*02),* defining Fy(a+b-), Fy(a-b+) and Fy(a+b+) as major phenotypes in Caucasian population. The Fy(x) phenotype, characterized by weak Fy^b antigen expression, is also relatively frequent (2–3.5%) in Caucasians and it is caused by the mutations c.265C>T and c.298G>A present in the *FY*02M.01* allele. In black individuals, the Fy(a-b-) phenotype is more common and is associated with a point mutation at nucleotide -67 (c.-67T>C) at the GATA-1 binding motif of the *FY*B* allele (*FY*02N.01*) that impairs the promoter activity and suppresses Duffy antigen expression in erythroid cells. Although much less frequently, mutations causing the Fy(a-b-) phenotype have also been reported in Caucasian individuals.
Aims: The aim of this study was to investigate the molecular basis of the Fy(a-b-) phenotype in a Caucasian donor carrying an apparently normal *FY*02* allele in heterozygosity with a *FY*02N.01* allele.
Methods: Duffy phenotyping was performed with ID/IAT-Cards using ID-anti-Fya and ID-anti-Fyb polyclonal antibodies (Bio-Rad). The *FY* genotype was determined by an "in-house" sequence-specific primer (SSP)-PCR method analyzing the *FY*A/FY*B* polymorphism, the presence of the -67T>C GATA mutation and the presence of the 265C>T mutation responsible for the Fy(x) phenotype. Sequence analysis of the *FY*02* allele was undertaken, previous *ACKR1* (*FY*) gene amplification (exons 1 and 2) using a GATA consensus-specific forward primer, to avoid *FY*02N.01* allele amplification. Sanger sequencing was carried out on an ABI PRISM 3130 Genetic analyzer.
Results: The donor's Fy(a-b-) phenotype was detected during extended blood donor antigen typing at the Blood Bank. *FY* genotyping is routinely performed in such cases, to investigate the presence of the *FY*02M.01* allele with a weak Fy^b antigen expression associated. The donor's *FY* genotype was found to be *FY*02/FY*02* (c.125A/A) by SSP-PCR and heterozygous for the -67T>C mutation in the promoter GATA motif, silencing one of the two *FY*02* alleles. As the 265C>T mutation responsible for the Fy(x) phenotype was excluded, we investigated the presence of other sequence alterations in the apparently normal *FY*02* allele. Sequence analysis revealed a deletion of a thymine at position 400 (c.400delT) in exon 2, leading to a frameshift and to the appearance of a premature termination codon (p.Cys134Valfs*13). This mutation has not been previously described.
Summary/Conclusions: We describe a case of a Caucasian blood donor with a Fy (a-b-) phenotype, resulting from the combination of a new *FY*02 null* allele, c.400delT (p.Cys134Valfs*13) and the *FY*02N.01* allele (c.-67T>C mutation in the promoter region), commonly found in black individuals. Adsorption/elution studies could not be performed in the available sample but will be undertaken in the next blood donation, to confirm the lack of Duffy protein expression in RBCs of this donor.

P-531

A NOVEL MUTATION IN AN APPARENT FY(A- B-) PHENOTYPE

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Background: In our region, Piemonte, the frequency of transfusion requesting a phenotype Fy(a-b-) is increasing. This phenotype is common in people of african origin, but rare in people of caucasian origin. The limited number of Fy(a-b-) is often dedicated to alloimmunized patients or to patients with sickle cell disease who are at greater risk to became alloimmunized. In this case the donor was of probable caucasian origin.
Aims: Our database encompasses nearly 25.000 donor phenotyped for Duffy, but we have an extremely limited number of donor of caucasian origin phenotyped as Fy(a-b-). We suspect that serological phenotyping as Fy(a-b-) of people of Caucasian origin may deserve an high grade of suspicion about the results.
Methods: A donor was phenotyped as Fy(a-b-) using multiple Methods tube test, gel test (BIO-RAD) and solid phase (Immucor). The DNA needed for genotyping was extracted using EZ1-Qiagen and subsequently tested with IDCOREXT(Grifols). The results were not clearly attributable to a known genotype, but the results could have been related to a novel mutation. The specimen was sent to a reference laboratory and sequenced (BLOOD Chip Service/Progenika-Grifols, GRIFOLS IH CENTER, S.Marcos Texas. USA).
Results: DNA sequencing of *FY* exons 1 and 2 and genomic DNA cloning and sequencing of *FY* exon 2 were performed. The genotype of the donor has been classified as *FY*A(762A),FY*B(265T,298A).* The genotype *FY*B(265T,298A)* encodes $Fyb+^w$ phenotypes. Variant allele *FY*A(762A)* to the best of our knowledge is unreported. This genotype has an NT change c.762G/A (AA change p.254Trp/Ter). Given that polymorphism c.762A introduces a premature STOP codon it seems likely that *FY*A(762A)* encodes a null phenotype.
Summary/Conclusions: We found a donor with an apparent phenotype Fy(a-b-) as determined by serological tests. Even if we did not find any discrepancies in the serologic phenotyping the donor was not of African origin. For this reason we decided to genotype the donor and found a novel mutation that could probably lead to a phenotype Fya null. The molecular analysis also revealed that the probable Fyb phenotype of the donor is a weak Fyb. In such cases the weak phenotype may be not detected as positive by the majority of reagents. It is not clear if such phenotype could lead to antibody production or transfusion reaction if transfused to people Fyb- at high risk of alloimmunization or already immunized. Donor with caucasian origin typed serologically as Fy(a-b-) should be tested by molecular genotyping.

P-532

IDENTIFICATION OF MUTATIONS IN POLISH ANTI-JRA OR ANTI-LAN PATIENTS AND THEIR RELATIVES

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Background: Anti-Jr^a and anti-Lan antibodies may cause hemolytic disease of the fetus or newborn (HDFN) as well as hemolytic transfusion reactions. Antigen-negative donors should be selected for immunized patients but such donors are extremely rare and difficult to identify with serological methods. Since 2012 when the genetic background of Jr^a and Lan was first described in Jr^a-negative or Lan-negative Caucasian individuals there appeared reports on multiple mutations in *ABCG2* and *ABCB6* genes.
Aims: Analysis of Polish patients with anti-Jr^a or anti-Lan and their relatives for the presence of polymorphisms commonly found in other Caucasian populations.
Methods: DNA was isolated from whole blood of a patient with anti-Jr^a, her neonate, father and aunt as well as from 8 patients with anti-Lan using NucleoSpin Blood Kit (Marcheney Suddenly GmbH). Real-time PCR was performed on LightCycler 480II (Roche) for the 3 null mutation in *ABCG2* gene (c.376C>T, c.706C>T, c.736C>T) and 3 null mutations in *ABCB6* gene (c.1867delins, c.1942C>T, c.2256 + 2T>G) using Taqman and SYBRGreen chemistry, respectively.
Results: In the patient with anti-Jr^a and her relatives the *ABCG2*01N.02.01* (c.706C>T) allele was detected. In the 8 patients with anti-Lan no mutations were found in all tested regions of *ABCB6*.
Summary/Conclusions: The mutation found in Jr^a-negative Polish patient is frequently detected in European individuals especially of Gypsy origin. The molecular background of Lan-negativity in the Polish patients requires further investigation through sequencing of the whole *ABCB6* gene.

P-533

A NOVEL DELETION MECHANISM IN GYPB ABOLISHES RED BLOOD CELL EXPRESSION OF THE U ANTIGEN IN A PROBAND OF AFRICAN DESCENT

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Background: S, s and U antigens are carried on the sialoglycoprotein glycophorin B (GPB). GPB is encoded by the *GYPB* gene. The S-s- phenotype, either U- or U+var, is mainly found among people of African descent. The S-s-U- phenotype is due to a *GYPB* large deletion while the S-s-U+var phenotype is caused by mutations involved in different exon 5 skipping found on two major alleles: *GYPB*P2* and *GYPB*NY*. In France, S-s- patients are systematically genotyped to know whether the high-prevalence U antigen is not or weakly expressed. In this study we report a S-s- patient of African descent being unexpectedly genotyped as homozygous for the *GYPB*s* allele.
Aims: We investigated *GYPB* in a patient of West African ancestry (Fulani ethnic origin) with a S-s- phenotype, who was predicted to be S-s+ from the MNS genotyping results.
Methods: Serological testing was performed by standard hemagglutination methods. Of note, U typing was carried out with an in-house high-titer anti-U reagent developed by a S-s-U- patient (*Del GYPB*). The *GYPB* gene was PCR-amplified from whole blood genomic DNA. The *GYPB* polymorphisms were determined by the Human Erythrocyte Antigen (HEA) BeadChip device (Immucor/BioArray Solutions, Warren, NJ, USA), by real-time PCR assays, and by standard genomic DNA sequencing. Finally, the full exonic coding sequence and flanking intronic region of *GYPB* gene was sequenced after exon-specific amplifications.
Results: Serology: the patient's RBCs typed M+N+S-s-U-; he was also found to show a second rare phenotype, R^NR^N (Sec-). The RBC antibody screen was negative. DNA: automated HEA BeadChip, real-time PCR assays and *GYPB* sequencing were consistent with a *GYPB*s/GYPB*s* (or *GYPB*s/Del GYPB*) genotype, without any mutation associated with *GYPB*P2* and *GYPB*NY* alleles. However, *GYPB* sequencing revealed that the sample was homozygous (or hemizygous) for a 5-bp deletion at the beginning of the first GYPB intron (c.37 + 4_8delAGTGA). This novel genetic basis very likely leads to a deleterious consequence for *GYPB* splicing, resulting in the lack of *GPB* transcripts. Consistently, the patient's red blood cells were unambiguously found to be S-s-U-.
Summary/Conclusions: We report here in a patient of West African origin a novel genetic background of *GYPB*, c.37 + 4_8delAGTGA, that appears to abolish *GYPB*s* allele expression. Out of the exceptional M^kM^k phenotype (*GYP*01N*), this novel allele *GYPB*s* (c.37 + 4_8delAGTGA) is the second molecular basis, after *GYPB* deletion (*GYPB*01N*), associated with S-s-U- phenotype. We suggest this allele to be officially named *GYPB*02N*, subject to the agreement of the ISBT Working Party on Red Cell Immunogenetics and Blood Group Terminology.

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SILENT KEL ALLELES IN JAPANESE

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Background: A rare K_o (Kell null) phenotype is lacking all 36 antigens in the Kell blood system. The incidence of K_o phenotype has been reported 0.003% in Japanese (2 in 63,000) which is similar to the Caucasian population (0.004%). The molecular basis of the K_o phenotype has been investigated, and more than 40 silent *KEL* alleles were reported. The majority of silent alleles are *KEL*02* background.
Aims: We report here molecular genetic analysis of the *KEL* gene in Japanese individuals with K_o phenotype.
Methods: K_o phenotype was screened from the Japanese blood donors for several years using a monoclonal anti-Ku (CBC-117) or anti-K14 (CBC-120) by an automated blood grouping system PK7300. Kell-related antigens were typed by standard tube tests. Genomic DNA was extracted from the blood samples and *KEL* gene was analyzed by PCR and sequencing.
Results: We collected 33 K_o blood samples with K−k−, Kp(a−b−), Js(a−b−), and K14−. No one had anti-Ku in the plasma. PCR and sequence analysis revealed that 11 individuals were homozygous for mutant *KEL* allele with a c.299G>C (p.Cys100-Ser) mutation (rs. 200268316). Two individuals were homozygous for *KEL*02N.24* allele having a c.715G>T (p.Glu239*) and one individual was homozygous for *KEL*02N.40* having a c.1474C>T (p.Arg492*). Five individuals were homozygous for novel *KEL* alleles with single nucleotide mutations, 4 individuals had a c.2175delC (p.Pro725 fs*43) and 1 individual had a c.328delA (p.Arg110 fs*79). Remaining 14 individuals were compound heterozygous with one of the above alleles. Among the 14 individuals, we identified 7 more new alleles with one or two mutations as follows: c.160delATinsCTCC (p.Ile54 fs*80), c.481A>T (p.Ile161Phe), c.[937G>A; 1073G>C] p.[Ala313Thr; Arg358Thr], c.997C>T (p.Gln333*), c.1414−1G>C (Alternative splicing), c.2120delC (p.Ser707 fs*17). Finally, we identified 12 different silent *KEL* alleles with *KEL*02* background from the 33 K_o individuals. Among the individuals, the *KEL* alleles with c.299G>C, c.2175delC, and c.328delA were major alleles with relative occurrences of 44%, 20% and 14%, respectively.
Summary/Conclusions: We identified 3 known and 9 new silent *KEL* alleles from the Japanese individuals with K_o phenotype. The silent *KEL* allele with the c.299G>C (p.Cys100Ser) was most predominant.

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MNS ANTIGEN MG, ENCODED BY GYPA*11 EXCLUSIVELY APPEARS AS POINT MUTANT OF GYPA*02 (N) WITHIN THE ZURICH AREA OF SWITZERLAND

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Background: The human MNSs blood group system is encoded by the two genes glycophorin A (*GYPA*) and glycophorin B (*GYPB*). Due to nucleotide substitutions, genetic hybrids and gene duplication and deletion events, the MNSs blood group system is considered as second in complexity to Rh. Mg, encoded by *GYPA*11*, is a low-frequency antigen located on *GYPA*. It has repeatedly been described as exhibiting a *GYP*(A-B-A) hybrid structure and including a C>A substitution at coding nucleotide c.68. Dating back to the 1960s, Mg had been reported to be found on both *GYPA* alleles, i.e. M and N, but with virtually no detectable expression.
Aims: Among others, routine MNSs blood group genotyping revealed one specific type of sero/genotype discrepancy, including several cases and involving *GYPA*11* in all of them. Mg is very rare, with higher incidences only reported for Swiss and Sicilians, reaching up to one Mg positive individual among 600. The observed absolute number of available Mg positive cases prompted us to (re)investigate Mg and its molecular background in detail.
Methods: MALDI-TOF MS based blood group MNSs genotyping interrogated c.59C>T of *GYPA* for MN, and c.143T>C of *GYPB* for Ss phenotype predictions. All genotyping results were compared to MNSs phenotypes, obtained by standard-serological methods. Originally discrepant phenotypes were reconfirmed on subsequent, independent samples. Genotyping was repeated using a commercially available PCR-SSP based method, including testing for *GYPA*11* (inno-train GmbH, Kronberg i.T., Germany). An exemplary number of *GYPA*11* positive samples and two individuals each of MMSS, MMss, NNSS, and NNss phenotypes were sequenced for *GYPA* from intron 1 (102 bp), across exon 2 and intron 2 (335 bp).
Results: MALDI-TOF MS based MN genotyping of 11.240 blood donors of the Zurich area in Switzerland delivered seven cases with M+N- serology, but a preliminary *GYPA*01/02* (MN) genotype. All genotype repetitions delivered Mg positive and final *GYPA*01/11* heterozygous results. No cases with M-N+ serology, a preliminary *GYPA*01/02* and final *GYPA*02/11* result, indicative of a Mg mutation located on *GYPA*01* (M), were observed. Alignments of the investigated sequence did not show any *GYPB* specific nucleotides on *GYPA*11* and exactly corresponded to the *GYPA*02* (N) allele, beside its specific c.68C>A point mutation. *GYPA*11* allele frequency was calculated from its appearance in MN heterozygous individuals and resulted in 0.136%. The expected overall frequency of Mg positive individuals resulted in one among 368 in the Zurich area of Switzerland.
Summary/Conclusions: Applying MALDI-TOF MS high throughput genotyping to 11.240 Swiss blood donors from the area of Zurich revealed seven Mg positive individuals with M+N- serotype, but a preliminary *GYPA*01/02* (MN) genotype. Serological retyping in these samples remained N negative, and preliminary genotype only became correctly identified as *GYPA*01/11* by subsequent PCR-SSP. We did not observe any *GYPA*11* specific point mutation located on a *GYPA*01* (M) allele. Controversially to several reports of Mg showing a *GYP*(A-B-A) hybrid structure, sequence alignments rather suggested presence of a simple point mutation, i.e. at c.68C>A located on *GYPA*02* (N) as causative for *GYPA*11*.

Platelet immunology

P-536

GENOTYPING OF HUMAN PLATELET ANTIGENS IN AN ADMIXED POPULATION

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Background: Human Platelet Antigens (HPAs) are polymorphic structures located on the platelet glycoprotein complexes and have been implicated in alloimmune disorders, including foetal and neonatal alloimmune thrombocytopenia, post-transfusion purpura and platelet transfusion refractoriness. The frequencies of HPAs vary between different populations. In Caucasians, HPA-1 is the most important antigenic system involved in platelet alloimmunity. The population of Argentina is composed predominantly by Caucasian Europeans admixed with Amerindians and Africans and the admixture degree varies among different social groups within a city. In this sense, the HPA polymorphism distribution occurring in our population is scarcely known.
Aims: The aim of this work was to investigate the genotype and allele frequencies of the HPA-1, HPA-2 and HPA-3 systems in individuals from two groups representing different social strata of the population.
Methods: Two sample cohorts of unrelated adults of both sexes from the city of Rosario, the third largest city located in the central area of Argentina, were studied. The first cohort (Group 1 [G1]) included 131 blood samples collected at a public health care center, where the assistance is supported by the provincial government and is intended for the low-income population. The second cohort (Group 2 [G2]) included 105 blood samples collected at a private clinical laboratory, where clinical tests are financed through insurance companies. HPA typing was performed by molecular strategies based on PCR-SSP and PCR-RFLP.
Results: HPA-1 genotype frequencies for G1 were: 1a/1a = 0.83, 1a/1b = 0.17, 1b/1b = 0.00 while for G2 were 1a/1a = 0.71, 1a/1b = 0.28, 1b/1b = 0.02. The allele frequencies found for G1 were: HPA-1a = 0.92, HPA-1b = 0.08 while for G2 were HPA-1a = 0.84, HPA-1b = 0.18. HPA-2 genotype frequencies for G1 were: 2a/2a = 0.73, 2a/2b = 0.26, 2b/2b = 0.02 while for G2 were: 2a/2a = 0.85, 2a/2b = 0.13, 2a/2b = 0.02. The allele frequencies found for G1 were: HPA-2a = 0.86, HPA-2b = 0.15 while for G2 were HPA-2a = 0.91, HPA-2b = 0.09. HPA-3 genotype frequencies for G1 were: 3a/3a = 0.38, 3a/3b = 0.47, 3b/3b = 0.15 while for G2 were: 3a/3a = 0.44, 3a/3b = 0.53, 3b/3b = 0.03. The allele frequencies found for G1 were: HPA-3a = 0.62, HPA-3b = 0.38 while for G2 was HPA-3a = 0.71, HPA-3b = 0.30. According to chi-square test, statistically significant differences were found for the HPA genotype distribution between both groups (HPA-1: $P < 0.02$; HPA-2: $P < 0.04$ and HPA-3: $P = 0.001$) and for the allele frequency (HPA-1: $P = 0.00138$; HPA-2: $P = 0.0476$; and HPA-3: $P = 0.0239$).
Summary/Conclusions: These results are consistent with our previous studies in erythrocyte blood group systems showing ethnic variability in the different social groups analyzed. A comprehensive study of the HPA polymorphism and allele distribution in our population will contribute to the organization of a regional registry of HPA-genotyped aphaeresis donors for a better management of alloimmunized patients.

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FAST, ACCURATE, & EASY REAL-TIME PCR HPA TYPING WITH LINKSĒQ™

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Background: The interaction of membrane-bound platelet-specific glycoproteins with the extracellular matrix plays a significant role in hemostasis. Human Platelet Antigens (HPA) found within these glycoproteins can stimulate production of antibodies in recipients of transfused platelets or in the developing fetus of mothers with incompatible HPA. As a result, platelet incompatibility is associated with various forms of thrombocytopenia, post-transfusion purpura and other blood disorders. The New Zealand Blood Service performs HPA typing on a pool of platelet donors in order to provide compatible transfusions where the need arises.
Aims: The molecular basis of most HPAs has been characterized as generally caused by a single-nucleotide polymorphism (SNP). HPA typing has typically been performed using PCR-SSP, a method that utilizes time-consuming post PCR analysis steps. The aim of this study was to evaluate the use of real-time PCR-based techniques in a transfusion laboratory setting.
Methods: We choose to evaluate the solution developed by Linkage Bioscience which consists of 24 reactions that identify both variants of 12 relevant SNPs located within HPA genes (HPA-1 through HPA-11, and HPA 15). Genomic DNA purified from 48 blood samples, previously genotyped for HPA-1,-2,-3,-4,-5 and -15 by our in house PCR-SSP method were used in this study as validation samples.
Results: Genotyping results of the validation samples were 100% concordant with typing obtained by PCR-SSP. The LinkSēq system overcomes the major challenges of HPA molecular typing by providing a robust and automated approach resulting in increased laboratory productivity and decreased turn-around time. The analysis is facilitated by SureTyper™ software which generates rapid typing results. With less than 10 min of hands-on set-up and no further operator intervention with the reagents, LinkSēq uses state of the art real-time PCR detection to provide complete molecular genotyping results in approximately 90 min. Further, since amplified products are never handled, the risk of laboratory contamination is significantly reduced.
The LinkSeq product was implemented by the New Zealand Blood Service Tissue Typing laboratory in late 2016 and to date has tested 749 DNA samples from 400 blood donors (with 349 donors being tested in duplicate). Concordance between the sample replicates was 100%. There were 24 occasions where the assay had to be repeated, giving a repeat rate of 3.2%. Occasionally a reaction peak was insufficient to trigger the software automatic allele call and a manual interpretation was required. This occurred most commonly with the HPA-3 (4.7%) and HPA-5 (1.2%) assays.
Summary/Conclusions: The LinkSēq system used in this study provides an effective, robust an accurate method for molecular HPA genotyping. With its minimal hands-on time workflow, it is also very easy to implement and offers a cost effective alternative to classical methods used in a transfusion laboratory setting.

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A NEW TYPING METHOD TO ASSESS HPA-1 TO-11 AND HPA-15 USING REAL TIME PCR

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Background: To date, 35 Human Platelet Antigens (HPA) have been identified, and are implicated in alloimmune platelet disorders including foetal/neonatal alloimmune thrombocytopenia, post-transfusion purpura, and multi-transfusion platelet refractoriness. Diagnosis of antiplatelet allo-immunization relies on the characterization of the specific alloantibody and the identification of the offending antigen. Up to date platelet genotyping is considered as the routine method for platelet antigen typing. Laboratory investigation for common HPA is no longer sufficient to evaluate maternal immunization or the potential for allo-antigen sensitization among high-risk patients.
Aims: In this work, we evaluated the robustness of a new typing technology developed by Linkage Biosciences on a given set of samples, some of them containing 1/ rare HPA antigens, or 2/Single Nucleotide Polymorphisms (SNP) located near HPA polymorphisms, and previously described as responsible of false genotyping results (Bertrand et al., *Transfusion* 2006, 2008, 2010, 2014; *Vox Sanguinis* 2013).
Methods: The LinkSēq™ technology is based on sequence specific amplification using SYBR® Green detection and melting curve analysis for specificity. The amplification and readout is done on a Real Time Instrument. Fluorescence intensities are automatically analyzed by the SureTyper software designed by Linkage BioSciences. Because of an extremely easy workflow, this technology is very well adapted for high throughput screening or emergencies, e.g. in a context of a mother and her severely thrombocytopenic newborn, 2 samples can be genotyped for HPA-1 to -11 and -15 in about 1.5 h. Twenty-seven samples were genotyped using the LinkSeq technology. They were all previously typed by Sequence Based Typing (SBT). One sample was duplicated in order to evaluate the reproducibility of the technology.
Results: Ten DNAs containing rare platelet antigens were typed (HPA-2ab (tested twice), HPA-4ab, HPA-6abw, HPA-7abw, HPA-8abw, HPA-9abw, HPA-10abw, HPA-11abw). All samples were correctly genotyped for the rare antigens, showing that PCR primers were correctly designed. One sample did not give a correct result for another antigen; however a cross-contamination between 2 samples was strongly suspected.
In addition, we genotyped 6 DNA samples carrying SNPs located near HPA-1, 3, 5 or 15. Sample #1 carried the rs36080296 SNP neat HPA-1; genotyping was HPA-1ab as expected. Sample #2 with the SNP rs5920 was correctly genotyped HPA-1aa. The SNP rs143860806 on Sample #3 did not affect the HPA-3ab genotyping. A novel SNP located near HPA-5 on Sample #4 (not yet recorded) does not modify the expected HPA-5aa genotype. Samples #5 which carries the SNP rs138465270 near

HPA-15 was correctly assigned HPA-15ab. The last sample carried the SNP rs199617851, which had no impact on the HPA-15ab genotype. This clearly demonstrates that PCR primers were correctly designed even for the most common platelet antigens HPA-1, -3, -5 and -15. Moreover, interpretation by SureTyper software was not affected. Finally, eleven DNAs with common typing were tested. We observed a 100% of concordance with the expected genotyping results.
Summary/Conclusions: These results on a restricted set -of rare samples demonstrates the value of the new HPA kits from Linkage Biosciences, providing a simple and fast alternative to PCR-SSP, allowing low and high throughput typing for common and rare HPA systems.

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A NEW DISCREPANCY OF HPA-3 GENOTYPING DUE TO A RARE HPA-27BW ANTIGEN IN A CONTEXT OF SEVERE NEONATAL THROMBOCYTOPENIA

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Background: Fetal/neonatal alloimmune thrombocytopenia (FNAIT) results from maternal alloimmunization against fetal platelet antigens. Due to the scarcity of phenotyping reagents in platelet immunology, most laboratories perform genotyping but a number of uncharacterized mutations and single nucleotide polymorphisms located near HPAs have been reported to cause false genotyping results.
Aims: Here, we report the case of a 28 year-old woman from Angola, third pregnancy/second delivery (one voluntary abortion) who gave birth to a severely thrombocytopenic boy (50.10^9/L) at 39 weeks of gestation. The father was from Congo. Twelve hours after delivery, the newborn's platelet count was 24.10^9/L and he was admitted into the intensive care unit even though no bleeding had been observed. He was given two platelet transfusions and IvIg. The baby's platelet count slowly rose and he was finally discharged in good health 16 days after delivery.
Methods: Platelet genotyping was performed by PCR-SSP (HPA Ready Gene Plus kit, Inno-Train) and sequencing (in-house method), and antibody detection using the MAIPA method.
Results: In the absence of any evidence of a non-immune etiology, tests were carried out to investigate this case of neonatal thrombocytopenia. HPA genotyping of parents and baby by PCR-SSP showed HPA-5b feto-maternal incompatibility. Using the MAIPA method, antibodies against HPA-5b were detected in the mother's blood confirming the diagnosis of FNAIT. Surprisingly, the newborn's genotype was found to be HPA-3bb while the mother was HPA-3aa and the father HPA-3ab (PCR-SSP). In order to investigate this, exon 26 of the integrin αIIb gene (GPIIb) was sequenced. The HPA-3 typing of the parents was confirmed, but the newborn was HPA-3ab not HPA-3bb: the "a" allele was not amplified by PCR-SSP. In fact, both mother and newborn also carried the rare HPA-27bw polymorphism (the αIIb-c.2614C>A mutation according to the International Nomenclature). HPA-27bw is close to HPA-3 and within the sequence of the PCR-SSP primer used to amplify HPA-3a which is why this allele was not detected in the newborn.
Summary/Conclusions: Rare platelet antigens should be investigated in absence of any non-immune etiology for fetal/neonatal thrombocytopenia coupled with the absence of evidence of feto-maternal incompatibility among the common antigens. This case also points up the importance of the localization of PCR primers used for HPA genotyping.

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MOLECULAR BACKGROUND OF THE LACK OF REACTIVITY OF HPA-1A ANTIGEN WITH MONOCLONAL SZ21 IN FACS – RESPONSIBLE FOR FALSE NEGATIVE RESULTS IN THE PREVFNAIT SCREENING PROGRAM

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Background: Reliable identification of women at risk of HPA-1a alloimmunisation (HPA-1a negative women) is crucial for screening programs for Fetal Neonatal Alloimmune Thrombocytopenia. This requires detailed knowledge of the background and implications of discrepancies between serological and DNA based methods, as well as understanding of the genetic mechanisms of *ITGB3* expression determining the antigenic epitopes.
Aims: Our aim was to determine: 1/the frequency of HPA-1a false-negative results of HPA-1a phenotyping by FACS with Sz21 MoAb used in a screening program recently performed in Poland; 2/the results of sequencing, cloning and expression of *ITGB3* gene fragment which control the formation of HPA-1 antigenic determinants in phenotypically HPA-1a negative woman with HPA-1a allele detected by TaqMan technology; 3/analysis of reactivity of mutated protein coated on beads with different monoclonal antibodies.
Methods: 15,204 pregnant woman were tested for HPA-1a by flow cytometry (FACS) using Sz21, a murine pseudo-specific HPA-1a monoclonal antibody, that binds to HPA-1a only in a concentration-dependent manner (Killie et al.) and by real-time PCR method with TaqMan technology (Ficko et al.). All HPA-1a negative woman (376) were verified by genotyping. In one case, the HPA-1a/1b genotype was identified. RNA from her platelets was isolated and used for cDNA synthesis followed by *ITGB3* cDNA amplification, sequencing, cloning and recombinant expression in insect cells. The protein variants were coupled to beads and analysed by FACS with different ITGB3 reactive moAbs.
Results: The frequency of false negative HPA-1a phenotyping by FACS using Sz21 antibody was 1/356 HPA-1a negative woman (1/15, 204 consecutive women). Sequencing of *ITGB3* in the examined case confirmed the heterozygosity at nucleotide 196, defining the HPA-1 allotype and revealed two heterozygous positions G175T and C176T in Exon2 in *cis* with the allele encoding the HPA-1a, reported earlier (as a part of NCBI Homo Sapiens Genomic Annotation Release 107 in 2015) together resulting in a C26F amino acid substitution in the mature protein. FACS analysis of beads coated with mutated protein ITGB3-F26-L33 confirmed that its reactivity with moAbs Sz21 antibody was completely abolished. It was also not recognized by MoAb 26.4 – specific for HPA-1a. The anti-GPIIIa antibodies Y2/51 and AP3 which recognize both normal ITGB3-L33 and ITGB3-P33 variants also showed reduced reactivity towards the mutated variant suggesting that the detected mutations influence their epitopes on this protein.
Summary/Conclusions: The frequency of false-negative results of HPA-1a phenotyping is ~3/1 000 HPA-1a negative woman. The results of our study confirmed that the lack of reactivity of HPA-1a antigen detected by FACS with monoclonal Sz21 was due to two recently reported adjacent SNPs in Exon2, which in the mature protein caused amino acid exchange in close proximity to the HPA-1 a/b polymorphism in Exon3 of *ITGB3*. Such results are important for extending the knowledge on molecular configuration of the HPA-1 epitopes which still is not fully known.

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NEW MOLECULAR BASIS ASSOCIATED WITH CD36 NEGATIVE PHENOTYPE

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Background: Glycoprotein CD36 comprises 472 amino acids and is expressed at the surface of various cell types, including platelets, monocytes and erythroid precursors. CD36 is also the receptor for several ligands (i.e. thrombospondin, collagen or fatty acids). However, absence of CD36 expression (CD36 NEG phenotype) seems asymptomatic, and is described in 0.3% of Caucasians, 3 to 11% of Asians and 2.5 to 7% of African Caribbean. Lack of CD36 expression exposes to the risk of immunization in case of pregnancy or platelet transfusion.
Aims: Our aim is to better characterize the defect in sickle cell patients that we identified of CD36 NEG phenotype, as part of the collaboration between the Sickle Cell Disease Reference Center, Henri Mondor Hospital in Creteil and E.F.S Ile de France.
Methods: CD36 NEG phenotype was firstly diagnosed by immunofluorescence microscopy and MAIPA (Monoclonal Antibody Immobilization of Platelet Antigen) performed on isolated platelets, and then confirmed by flow cytometry (FCM). FCM was run in parallel for patients' isolated platelets and monocytes, in order to characterize the subtype (I or II) of the defect. Fifteen exons of the CD36 gene were subsequently sequenced by Sanger method, to better define the underlying molecular basis.
Results: 4 sickle cell disease patients were found of 36 NEG phenotype among patients studied in the Laboratory in Creteil over the last year. For 3 of them, further molecular characterization identified a T975G mutation leading to a stop codon in

exon 10. This mutation has already frequently been described in the African Caribbean CD36 NEG subpopulation. Interestingly, for one patient we identified for the first time a new molecular defect underlying the phenotype of interest. In this patient, two adenines are replaced by one Guanine in exon 4 (d.a367- a368; i.g367) leading to a frameshift responsible for a stop codon in position 76. Furthermore flow cytometry analysis of CD36 antigen expression done in platelets and monocytes shows neither platelets nor monocytes expression of CD36 according to the type I deficiency.

Summary/Conclusions: In this study we report for the first time a new molecular basis of the CD36 gene associated with a type I deficiency. To date, the potential functional impact of CD36 NEG phenotype in the African Caribbean population is still unknown, but a positive selective pressure for this phenotype, mediated by some yet uncharacterized infectious agents, is an hypothesis and could explain the increased frequency of the defect in some population.

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Abstract has been withdrawn.

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PLASMAPHERESIS IN COMBINATION WITH INDIVIDUALLY SELECTED PLATELET CONCENTRATES AS SECOND LINE OF THERAPY OF REFRACTORINESS TO PLATELET TRANSFUSIONS

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Background: Multiple transfusions of platelet concentrates (PC) in hematological patients (>20) are a risk factor for alloimmunity and refractoriness to PC transfusions caused by allosensitization of the recipient by donor blood cells Human Leukocyte Antigen (HLA) and Human Platelet Antigen (HPA). Refractoriness can also be caused by autoimmune cytolysis and non-immune factors. It is indicated to select compatible pairs "donor-recipient" for alloimmunized patients using individual cross-matching tests, allowing detection of the presence of circulating alloantibodies in the patient's serum. In cases with multiple alloimmunity individual selection may be difficult or impossible, the method of choice is applying plasmapheresis procedures (PPs) to eliminate circulating alloantibodies and circulating immune complexes.

Aims: To assess the efficacy of PPs in combination with the transfusion of individually selected cross-matching PCs in patients with refractoriness to plasma transfusion.

Methods: From October 2015 till January 2016 11 PC transfusion refractory patients have been treated in the center's clinics. Among them there were 2 patients with aplastic anemia (AA) and 9 patients with acute myeloid leukemia (AML). Additionally, 6/11 patients showed febrile non-hemolytic transfusion reactions (FNR) after transfusion with individually cross-matching plasma units. Considering inefficiency of the individual selection, it became necessary for these patients to perform additionally PPs. In total 65 PPs have been done with the scope of replacing half of circulating plasma volume (CPV) using the blood separator PCS2 (Haemonetics). On average 6 procedures (from 2 to 11), with an interval of 3 days (from 0 to 14 days) have been performed. Circulating blood volume was replenished with albumin, fresh frozen plasma and physiological solution. All patients received individually selected cross-matched PC transfusions on the day of PP. The efficacy of PPs with individually selected PC transfusions was assessed by Absolute Platelet Increment (API) and Corrected Count Increment (CCI), the level of circulating alloantibodies, probability of donor-recipient matching, as well as by decrease of adverse reactions to transfusions before and after performing PPs. Cross-matching and antibody activity in relative units (RU) was assessed with a Galileo-Neo (Immucor) analyzer.

Results: 10/11 patients receiving PP treatment in combination with individually selected PC transfusions showed increased API/CCIs, decreased circulating alloantibody activity and increased probability of donor-recipient matching against former transfusions without additional PP treatment. However, in 2 patients (AA, AML) adverse reactions persisted. These patients then received transfusions of platelets in Platelet Additive Solution (SSP+), which facilitated mitigation of the reactions. In one patient with AA inefficiency of therapy PP treatment plus individual cross matching selection was noted, in this case probability of autoimmune cytolysis of platelets, as well as increased intake syndrome cannot be ruled out.

Summary/Conclusions: In case of inefficiency of individual cross-matching PC selection in patients with refractoriness to platelet transfusions, PPs significantly improved the transfusion efficacy. Performing PPs in combination with individually selected PC transfusions decreased circulating alloantibodies activity, increased probability of donor-recipient matching, increased API and CCI, which significantly improved clinical efficacy and immunological safety of PC transfusions.

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CORRECTED COUNT INCREMENT (CCI) IN EVALUATION OF EFFICACY OF PLATELET TRANSFUSION THERAPY IN PATIENT WITH HPA ALLOANTIBODIES

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Background: A failure to reach posttransfusion platelet increment is called platelet refractoriness. This can be caused by immune and nonimmune factors. In immune platelet refractoriness HLA and/or HPA antibodies are the major cause. For evaluation of platelet transfusion efficacy the corrected count increment (CCI) is the most widely used. Timing of a posttransfusion platelet count is important for an interpretation of the CCI. A low CCI at 1 h after transfusion (CCI-1 h less than 7,5-5) is suggestive of immune causes, whereas a reduced CCI at 24 h (CCI-24 h less than 4,5) following a normal CCI-1 h is more suggestive of nonimmune clinical factors.

Aims: Our aim was to evaluate transfusion therapy efficacy.

Methods: Platelet specific antibodies were examined using platelet immunofluorescence test (PIFT) and LIFECODES Pak Lx assay [IMMUCOR]. HLA antibodies were examined using standard complement-dependent lymphocytotoxicity test (CDC – NIH) and ELISA technique LIFECODES QuikScreen [IMMUCOR].

Results: A 51-year old woman who had previously had one child, was diagnosed with acute myeloid leukemia (AML) and accepted to our institution for a treatment. Screening for platelet and HLA antibodies was carried out shortly after her admission. PIFT test was positive (1+), HLA antibodies screening was negative. Because of severe thrombocytopenia the patient was repeatedly transfused with platelets. According to CCI-1 h platelet transfusions seemed to be successful, CCI-1 h ranged between 4.32 and 26 with median 11. Nevertheless platelet count of the patient decreased after platelet transfusion and dropped within 24 h to very low counts even to zero. CCI-24 h ranged between 0 and −4.86. The primary sample was further investigated and anti-HPA-1a and anti-HPA-5b alloantibodies were detected. Thereafter the patient was transfused with apheresis platelets from HPA-1a, HPA-5b negative donors. When AB0 compatible HPA-1a, HPA-5b negative platelets were transfused, the CCI-1 h ranged between 6.48 and 32.4, median 13.8, CCI-24 h ranged between 1.62 and 15.66 with median 5.94 and kept the platelet count of the patient in safe numbers (around 20×10^9/L).

Summary/Conclusions: According to our experience with platelet transfusion therapy efficacy monitoring in patients with HLA antibodies, the CCI measured 1 h after transfusion (CCI-1 h) has been an appropriate evaluating factor. In this case of HPA alloantibodies CCI-1 h failed to demonstrate refractoriness despite persisting very low platelet count. Although CCI-24 h is commonly considered to be an indicator of nonimmune causes of refractoriness, in this case CCI-24 h helped to reveal immune caused refractoriness (due to HPA alloantibodies) and to accurate suitable transfusion therapy.

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EVALUATION OF PLATELET CROSS MATCHING, MANAGING SUSPECTED PLATELET REFRACTORY: A CASE REPORT

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Background: Cross-match-compatible platelets are used to support thrombocytopenic patients who are refractory to platelets. Most platelet transfusions are administered as a pool of six to ten random donor platelet concentrates. As HLA or platelet-specific antibodies develop, post-transfusion platelet increments diminish due to immune destruction. This case represents the effectiveness of cross-match-compatible platelets in a new born baby who had consistently been refractory to platelets from random donors.
Aims: Case Report: A premature delivered female baby with complaint of thrombocytopenia, leukopenia and repeated anemia was admitted in intensive care. Several random donor platelets were transfused but response was unsuccessful. Patient serum was then cross matched with multiple random donor platelets to identify and issue compatible platelets. Compatible units in Solid phase were transfused and observed for any refractoriness. Transfusion with platelets from crossmatch-compatible donor immediately gave good results.
Methods: Solid-phase red cell adherence method was used for platelet cross-matching on NEO (Automated immunohematoanalyzer). Patient serum was cross matched with potential random donor platelet units. IgG coated indicator cells in SPRCA method detected antibodies directed against platelet-specific HLA, or HPA antigens. SPRCA method provided a feasible and effective alternative to HLA matching as a means of donor selection for refractory platelet recipients. The speed and simplicity of this method may allow most hospital laboratories to perform platelet crossmatching before routine platelet transfusions. The corrected count increment was used to monitor the effectiveness of each platelet transfusion.
Results: Statistically significant improvements were found in the mean corrected count increment when comparing cross-match-compatible platelets with randomly selected and incompatible platelets Compatible platelet transfusions were associated with a good response in the presence of clinical factors or allo-immunization, along with good responses in CCI and PPR value using statistic formula. Both the CCI (Corrected Count Increment) and the PPR (Percent Platelet Recovery) are determined shortly after transfusion usually 10 – 60 min. A CCI greater than 7,500 platelets x m2 BSA/μl or a PPR greater than 20% are considered acceptable.
Summary/Conclusions: Patient underwent of platelet transfusion due to sudden falling of platelet from 269,000/μl to 15,000/μl within 4 days. Randomly selected units were given initially but statistically significant improvements were found in the mean corrected count increment when comparing cross-match-compatible platelets with randomly selected and incompatible platelets. Compatible platelet transfusions were associated with a good response from 26,000/μl to 235,000/μl, while with randomly uncross matched platelets there was no any improvement in a period of 24 days with 8 units of RDPC. Improvement of platelets in the presence of clinical factors or allo-immunization was calculated in Corrected Count Increment (CCI) and Percent Platelet Recovery (PPR) value using standard formula. Both the CCI and the PPR are determined shortly after transfusion usually 10–60 min. A significant changes in CCI i.e. 11,843 platelets × m^2 BSA/μl and in PPR i.e. 10% was observed.

Granulocyte immunology

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HNA-2 PHENOTYPE FREQUENCIES IN THAI BLOOD DONORS

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Background: Antibodies specific to human neutrophil antigen (HNA), especially HNA-2 is implicated in various conditions including neonatal alloimmune neutropenia, febrile transfusion reactions and transfusion-related acute lung injury. Information regarding the distribution of HNA-2 phenotype frequencies in Thai populations remains unknown.
Aims: We aimed to investigate the HNA-2 phenotype frequencies in Thai blood donors and to compare the relationship of sex and age to HNA-2 expressions on neutrophils.
Methods: EDTA blood samples of 200 unrelated healthy Thai blood donors, 150 males and 50 females with ages ranging from 20 to 57 years were included. Polymorphonuclear cells (PMNs) were isolated and stained with two monoclonal antibodies specific to human CD177 (MEM-166) and CD45. The percentages of HNA-2 expression were analyzed by flow cytometry and FlowJo software.
Results: Among 200 donors, the frequencies of HNA-2 phenotype frequencies were 0.995 and HNA-2 null phenotype (<5% antigen expression) was found only in one male donor. The percentages of antigen expressions in women (71.7 ± 15.5%) were higher than in men (64.6 ± 18.8%). Moreover, there was no significant difference between age and HNA-2 antigen expression.
Summary/Conclusions: This study is the first report of HNA-2 antigen frequencies in a Thai population, which will be helpful to predict the risk of alloimmunization to HNA-2 and to recruit granulocyte panel donors to screen HNA-2 antibodies.

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EVALUATION OF LABSCREEN MULTI IN THE DETECTION OF ANTIBODIES AGAINST THE HNA-3 SYSTEM

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Background: Antibodies directed to human neutrophil antigens 3a (HNA-3a) have been involved in fatal transfusion-associated acute lung injury (TRALI) reactions. For the HNA-3a antibody detection, the gold-standard method is the Granulocyte Agglutination Test (GAT); however, it is a time-consuming methodology. The new generation LABScreen-MULTI assay (LSM) kit (One Lambda, Inc.) is suitable for high-throughput testing and automating, using microbeads coated with HLA and all HNA antigens, including HNA-3a and -3b.
Aims: Considering that the LSM is a recently developed commercial platform, this study aimed to evaluate its ability to detect anti-HNA-3a and -3b.
Methods: Thirty-six samples, tested by the LSM (Lots 005 and 007), were included in the study, comprising 13 pregnant women, 12 multiparous blood donors, 4 alloimmunized patients with multiple antibodies, and 7 mothers whose newborns had neonatal neutropenia. The cutoff value was set to 5 NBG (Normalized Background Ratio) for the HNA-3a and -3b beads. Results of the LSM were confirmed by the HNA-3 genotyping and by the GAT, using a panel specific for HNA-3 antigens.
Results: LSM screening resulted in 31 samples with positive results for 3a and/or 3b beads, and 5 samples with negative results. 28/31 samples were considered false-positive because they presented negative results in the GAT and, in some cases, also presented discordant results with the genotyping. The false-positive reactions were observed on 3a and -3b beads, and in 10 samples occurred for both beads concomitantly. 24/28 false-positive samples presented NBG of 5 to 15 and Mean Fluorescence Intensity (MFI) values below 1,000; and 4/28 presented high NBG values, between 20 and 80, and MFI values, between 1,150 and 3,900. 3/31 samples were considered true-positive, and the antibodies were confirmed by positive results in 1 sample for anti-HNA-3a, and in 2 samples for anti-HNA-3b in the GAT. It is important to highlight that the anti-HNA-3a sample had a false-positive result with 3b bead (NBG = 17.6, MFI = 1,711), and 1 sample with anti-HNA-3b presented a false-positive result with 3a bead (NBG = 83, MFI = 4,313). False-negative results in the LSM were observed in 5 samples, since anti-HNA-3b was detected by the GAT, and their results were compatible with the HNA-3 genotyping.
Summary/Conclusions: HNA-3 antibodies are highly agglutinating, therefore, the GAT was chosen as a parameter to evaluate the results obtained in the LSM. Anti-HNA-3 was confirmed by the GAT in only 3/36 (8.3%) samples. False-negative results were observed just for the 5/36 (13.9%) samples tested with Lot 005. Considering that most samples (28/36 – 77.8%) presented false-positive reactions, we suggest a careful analysis of the results presenting an NBG variation from 5 to 15 and MFI less than 1,000. The analysis of the discordant results between the GAT and the LSM lead us to two possible conclusions: (i) Really false-positive results in the LSM due to inconsistencies with genotyping; and (ii) False-negative results in the GAT due to low titre and/or low agglutinating capacity. These antibodies cannot be disregarded in view of their possibility to induce TRALI by directly binding to the pulmonary endothelium.

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PREVALENCE OF LEUKOCYTE ALLOANTIBODIES IN BRAZILIAN MULTIPAROUS BLOOD DONORS

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Background: Leukocyte alloantibodies (anti-HLA class I, class II, and anti-HNA-1, -2 and -3) present in blood products are known to be responsible for antibody-mediated transfusion-related acute lung injury (TRALI), an important cause of transfusion-associated morbidity and death. Preventive strategies are still a matter of debate and prevalence studies of antibodies involved in different populations are a key part of the decision-making.
Aims: To assess the prevalence of leukocyte antibodies in blood donors of south-eastern Brazil.
Methods: Blood samples were collected from 467 multiparous blood donors (with two or more pregnancies). Antibody screening was performed using the LABScreen® Multi (LSM) kit (One Lambda Inc, Lot 7) comprising all HNA antigens and HLA class I and II, and the granulocyte agglutination test (GAT). Positive or inconclusive cases were investigated by white cell immunofluorescence test (Flow-WIFT) and HNA genotyping to confirm the antibody specificity. For the GAT and Flow-WIFT, a panel with at least 3 donors including all HNA antigens was used. The cutoff values established for the LSM were 5 normalized background (NBG) ratio for the HNA-1, -3, -4 and -5 systems; 20 NBG for the HNA-2 system; and 10 NBG for the HLA class I and II. For Flow-WIFT, a MFI 2-fold higher than the negative control serum was considered positive.
Results: Leukocyte alloantibodies (anti-HLA and/or anti-HNA) were detected in 211/467 multiparous blood donors, resulting in an overall alloimmunization rate of 45.2%, comprising 42.6% (199/467) of anti-HLA and 4.9% (23/467) of anti-HNA antibodies. With respect to the specificity of HLA antibodies, 32.8% (153/467) had anti-HLA class I, 24.4% (114/467) anti-HLA class II and 14.6% (68/467) class I and II. Among the donors with HNA alloimmunization, 47.8% (11/23) had concomitant anti-HLA antibodies. Regarding the specificity of HNA antibodies we found 7/23 anti-HNA-1a (NBG variation: 9.3–39), 1/23 anti-HNA-1c (NBG: 5.2), 3/23 anti-HNA-2 (NBG: 28–36), 2/23 anti-HNA-3a (NBG: 14–161), 5/23 anti-HNA-3b (NBG: 9–25), 3/23 anti-HNA-5a (NBG: 6–9) and 02/23 anti-HNA-5b (NBG: 11.3–16). False positive reactions (discordant with donor HNA genotyping) were identified in 36/467 (7.7%) samples. An MFI between 580 and 8,000 was observed in the positive reactions. Only 9/23 positive samples in the LSM showed reactivity in the GAT, including 1/2 samples with anti-HNA-5b.
Summary/Conclusions: The alloimmunization rates found in this study were the highest among those reported in the literature: 42.6% (199/467) for anti-HLA and 4.9% (23/467) for anti-HNA antibodies. This finding may be attributed to the high miscegenation rates in the Brazilian population, highlighting the importance of the implementation of leukocyte alloantibody screening in multiparous blood donors. Another peculiarity of this population is the higher prevalence of anti-HNA-3b antibodies: 21.7% (5/23) comparing to anti-HNA-3a [8.7% (2/23)]. Through the LSM we were able to detect new and rare specificities of HNA antibodies, such as the five cases of anti-HNA-3b and two possible anti-HNA-5b, not yet described in the literature.

P-550

MIR-146A NEGATIVELY REGULATES DECTIN-1-INDUCED INFLAMMATORY RESPONSES IN HUMAN THP-1 MACROPHAGES

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Background: With the growing number of immunosuppressive population in the recent decade, the incidence of life-threatening invasive *Candida* infections has increased dramatically. Innate immune system including neutrophils, monocytes, macrophages and dendritic cells constitute the first line of host defense against *Candida* infection. Dectin-1 is the critical sensor for β-glucan from *Candida* that could lead to the activation of innate immune responses. MicroRNAs (miRNAs) are small non-coding RNAs which play crucial roles in regulating innate immunity.
Aims: To determine the functional role of miRNAs in inflammatory response dependent on the activation of Dectin-1 pathway.
Methods: Profiling miRNAs expression of THP-1 cells induced by the interaction between Dectin-1 and CaIG were performed with miRNA microarrays and qRT-PCR validation. qRT-PCR and ELISA were used to detect the mRNA and protein expression of IL-6 and TNFα. Flow cytometry was used to analyze the protein expression of Dectin-1. To determine the activation of spleen tyrosine kinase (Syk), phosphorylation of Syk was assessed by western blot. To explore the activation of nuclear factor kappa B (NF-κB),the phosphorylation and degradation of IκB-α were detected by western blot, and nuclear translocation of NF-κB p65 was observed using confocal microscopy. Hsa-miR-146a mimics, has-miR-146a inhibitor and negative control were transfected into THP-1 cells using Lipofectamine 2000.
Results: In the present study, we found that insoluble β-glucan from the cell wall of *C. albicans* (*Ca*IG) could increase the production of IL-6 and TNFα through Dectin-1-Syk-NF-κB and p38MAPK pathway. MiRNAs profiles detected in THP-1 cells treated with *Ca*IG revealed that miRNA146a expression level increased. The interaction between Dectin-1 and *Ca*IG resulted in long lasting increase of miR-146a expression dependent on Dectin-1-Syk-NF-κB, p38MAPK, contrasting with a rapid and transient increase of IL-6 and TNFα. Overexpression of miR-146a significantly suppressed the production of IL-6 and TNFα. MiR-146a inhibited the activation of NF-κB triggered with *Ca*IG via Dectin-1.
Summary/Conclusions: Our data suggest that miR-146a may play the potent negative feedback regulator in inflammatory response following Dectin-1 stimulation.

Fetal-maternal immunology

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ANTI-HPA-1A ANTIBODY ACTIVITY AS A PREDICTING FACTOR OF THROMBOCYTOPENIA RISK IN THE NEWBORN

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Background: In order to identify women at risk of fetal/neonatal alloimmune thrombocytopenia (FNAIT) we introduced the PREVFNAIT screening program for HPA-1a antigen typing in Poland. All HPA-1a negative women were tested for anti-HPA-1a and – in positive cases – for antibody titer and activity.
Aims: The aim of this study is to present the results of correlation between antibody activity/level in plasma of pregnant women and platelet count in the fetus/newborn and to evaluate the positive and negative predictive values (PPV and NPV, respectively) of such testing. In addition we analyzed the antibody level in a samples collected before introduction of IVIG and in the subsequent samples.
Methods: The study was performed in 48 HPA-1a negative pregnant women with anti-HPA-1a detected in MAIPA. Plasma samples of 11 women were tested in the 16, 28, 32 and 38 weeks of gestation and 6 weeks after delivery and in 37 cases at least once during or after pregnancy. Quantitation of antibodies was performed in MAIPA using NIBSC anti-HPA-1a standard (03/152) according to Bertrand, Blood, 2011. The level of antibodies was correlated with the platelet count in the fetus (n = 5) or in the newborn of non-treated women (n = 20). Moreover, antibody levels were measured during IVIG therapy and after the delivery in 46 samples from 13 women.
Results: 1/Pearson's correlation coefficient for antibody level in plasma of pregnant women collected before IVIG administration or before delivery (in untreated cases) with the platelet count in the fetus/newborn is [−0.71]. Antibody activity (mean±SD; number of cases)/platelet count in the fetus/newborn: 39.17 ± 21.6 IU/ml (n = 7)/<50 × 10^9/l; 1.71 ± 1.81 IU/ml (n = 3)/50–150 × 10^9/l; 4.11 ± 5.30 (n = 15)/>150 × 10^9/l 2/Analysis of the anti-HPA-1a antibody level during IVIG treatment. In 4 treated women antibody level decreased from mean 53.06–6.22 IU/ml – the babies were born with no signs of bleeding and with no or with mild thrombocytopenia (239 × 10^9/l, 287 × 10^9/l, 124 × 10^9/l, 109 × 10^9/l respectively). In 1 woman antibody activity decreased from 47.81–6.67 IU/ml, the child had mild thrombocytopenia (100 × 10^9/l). In all women antibody activity increased immediately after delivery (to ~20.3 IU/ml). 3/For anti-HPA-1a activity cut-off set on 20 IU/ml: PPV of our method is 100% and NPV is 90%.
Summary/Conclusions: 1/The preliminary results suggest that the severity of thrombocytopenia in the fetus/child of HPA-1b/1b woman may be predicted by the level of anti-HPA-1a in maternal plasma. However, more studies are necessary to determine the predictive value and the cut-off in our laboratory. 2/PPV and NPV of our method seem reliable in establishing the risk of severe thrombocytopenia in fetus or newborn of HPA-1a negative woman with corresponding antibodies. 3/IVIG administration during the pregnancy influence anti-HPA-1a level and leads to increase of fetal platelets.

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THE DETECTION OF MATERNAL HPA-1A ANTIBODIES AND THEIR ROLE IN PREDICTING THE SEVERITY OF FNAIT: A SYSTEMATIC REVIEW

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Background: In Caucasians, fetal/neonatal alloimmune thrombocytopenia (FNAIT) is most commonly due to maternal human platelet antigen (HPA)-1a antibodies that may result in severe bleeding complications. HPA-1a typing and subsequent screening for anti-HPA-1a antibodies in HPA-1a negative women is one option to identify first pregnancies at risk. However, there is no agreement on optimal follow-up and intervention in immunized pregnancies, neither in first pregnancies identified by screening, nor in subsequent pregnancies. The prognostic potential of maternal HPA-1a antibody level, to differentiate between severe and non-severe thrombocytopenia in fetuses and newborns, has been studied during the last decade, but a consistent approach has not yet been determined.

Aims: The aim of this systematic review was to examine whether the maternal antibody level to HPA-1a could be used as a non-invasive technique to identify high-risk pregnancies with FNAIT.

Methods: The electronic databases MEDLINE, EMBASE and the Cochrane Library from 1946 to January 12, 2016 were searched. Original studies that included five or more pregnant women with pregnancies screened for or at risk for HPA-1a-induced FNAIT were included. The final studies included for assessment were studies with complete data sets which had examined the association between HPA-1a antibodies and fetal/neonatal outcomes. Before assessment the studies were categorized into two groups according to recruitment strategies; screening of unselected pregnancies and samples analyzed from known or suspected FNAIT patients.

Results: Four prospective studies reported results from screening programs and 10 studies focused on suspected FNAIT. There were several limitations in the quality of studies included. Antibody testing was performed at various times during pregnancy and several laboratory methods were used for antibody detection. Eight of the 14 studies used the monoclonal antibody immobilization of platelet antigen assay and identified a statistically significant relationship ($P < 0.05$) between HPA-1a antibody level measured either during the third trimester or at delivery (for unselected pregnancies) or during the second trimester (for pregnancies at risk) and the neonatal platelet count.

Summary/Conclusions: This review demonstrates that the HPA-1a antibody level has the potential to be used as a predictor for the severity of FNAIT.

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THE POLISH-NORWEGIAN HPA-1A NEGATIVE BIOBANK FROM PREGNANT WOMEN

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Background: HPA-1a platelet antigen is responsible for the majority of fetal/neonatal alloimmune thrombocytopenia (FNAIT). The organization of a biobank for the collection of biological samples and clinical data from HPA-1a negative pregnant women and their relatives is an important starting point for the identification of the complex mechanism and prediction markers of the disease.

Aims: to organize a small-scale semi-automatic biobank of HPA-1a negative pregnant women recruited from the whole Poland, their partners and neonates done as part of the HPA-1a screening program "PREVFNAIT" which is currently performed by the Institute of Hematology and Transfusion Medicine (IHTM) in Poland and University of Tromsø The Arctic University of Norway (the Project Agreement No. Pol-Nor/203111/69/2013).

Methods: HPA-1a negative pregnant women who signed the informed consent were registered into the clinical database (Optimed) and received a unique case number. From each woman, her partner and neonate (cord blood) samples of whole blood and DNA were collected. Plasma samples from each woman were collected when the woman entered the study and in the follow-up at 16–20, 28, 32, 38–40 weeks of pregnancy and 6 weeks after delivery. These samples are in parallel collected in Polish and Norwegian biobanks. The Norwegian biobank additionally contains cryopreserved PBMC samples. The samples were stored in 2D-barcoded cryopreservation tubes with a manually pasted label. Each visit, each kind of material and each relative had its own code added to the unique case number and printed as a barcode on the label. We developed a procedure that combined the computer system for archiving (ARCA) samples with these special labels. Before putting tubes in a barcoded rack, their positions were scanned and recorded in ARCA system using the 2D and the label barcodes. Full racks were verified electronically by 2D scanning. All racks were organized into boxes and kept in −80°C, The Norwegian part of the biobank is successively transported to Norway.

Results: After 3 years the PREVFNAIT Polish-Norwegian biobank has collected 558 whole blood and DNA samples from HPA-1a negative pregnant women, 545 from their partners, 373 from their neonates and 2,045 plasma samples from the immunized or non-immunized HPA-1a negative mothers. The Norwegian PBMC biobank contains 1,002 samples from all HPA-1a negative, *HLA-DRB3*01:01* positive women and from about 200 *HLA-DRB3*01:01* negative women.

Summary/Conclusions: The PREVFNAIT biobank has been constituted as the first collection of time-course samples from HPA-1a negative Polish population being designed for further researches of FNAIT disease.

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PLACE OF PLATELET IMMUNOLOGY WORKSHOPS IN THE FNAIT MANAGEMENT: TOWARDS A PRECISION MEDICINE AND TRANSLATIONAL RESEARCH

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Background: Fetal and neonatal alloimmune thrombocytopenia (FNAIT) occurs in 1:10,000 live births in Caucasians but the frequency is not really known in other populations. Serological and molecular Human Platelet Antigens (HPA) genotyping tests are carried out to investigate and conclude to FNAIT diagnosis. Since 1935, the International Society of Blood Transfusion (ISBT) has the aim of facilitating knowledge about transfusion medicine to serve the interests of donors and patients. Platelet Immunology Workshops (WS) are organized by ISBT in order to improve the quality of platelet antigen and antibody diagnostic and to promote research. They are crucial for the development and improvement of platelet immunohaematology in the world as many genotyping and serological tests are not yet standardized.

Aims: The aim of this study is to pinpoint the organisational problems and ethical questions encountered by members of the WS in the context of precision medicine and translational research in FNAIT management.

Methods: This work was carried out through two complementary approaches combining an analysis of all the WS reports elaborated by the organizers and scientific

articles related, and a survey with 7 Platelet Immunobiology Working Party (PIWP) members chosen for their very long participation to WS to collect information on their motivations, on organisation and ethical points (for instance place of patient, consent, immigration restrictions).
Results: WS reports and scientific articles related sum up information about exercises results on serology and HPA genotyping to evaluate and compare laboratory results and to elaborate technical and scientific recommendations for future WS. Moreover, organisational questions were raised especially the increasing number of laboratories and samples availability for each lab, immigration restrictions. . .Survey highlights different motivations of PIWP members participation –(i) professional including patients care and research –(ii) personal as education, collaboration and knowledges sharing. Regulatory and ethical questions emerged and were differently expressed and appreciated according to PIWP members such as patient place and consent collection, infectious status of samples.
Summary/Conclusions: Platelet immunobiology is of major clinical importance and clinical decisions are guided by laboratory results. This study brings to light ethical and organisation problems. In the future, removing blocking points and taking account ethical questions will help WS organisation which is in the heart of precision medicine and translational research. This reflexion is extremely important with the development of new technologies such as Next Generation Sequencing. In that context, a new survey will be conducted among PIWP members or WS participants thanks to items already identified in that qualitative study to appreciate more deeply particularly motivations and ethical questions.

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A NEW APPROACH TO MANAGE NEONATAL ALLOIMMUNE THROMBOCYTOPENIA (NAIT) USING HPA GENOTYPING FROM BUCCAL SWABS

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Background: The HPA Beadchip genotyping kit (CE-IVD BioArray Solutions, Immucor) is a molecular test to detect 22 human platelet antigens (11 polymorphisms) in a single multiplex DNA assay, a technology which was implemented since 2010 in our laboratory highly experienced.
Aims: In a former study carried out in 2015, we found that HPA genotyping was 97.6% concordant when performed either from blood samples (EDTA) or from buccal swabs (n = 189 patients). Although the current genotyping protocol required a DNA concentration ranging from 10 to 80 ng/µl, this technology was robust enough to genotype samples with DNA concentration below 10 ng/µl. Moreover, under 1 ng/µl, HPA genotyping results were correctly interpreted in 90% of cases. However, we did not focus on the swab conditioning methods and their implications in the yield of DNA recovery and thus on reliability of the HPA genotyping results.
Methods: To answer these questions, 95 DNA samples from blood and buccal swabs were genotyped and compared, by taking into account more particularly swabs conditioning (dry cotton, cotton in gel matrix or in medium preserving virus or bacteria). Two CE marked *in vitro* diagnostics techniques were used for DNA extraction: i) automated extraction with the MagNA PURE Compact Nucleic Acid Isolation Kit I (Roche Diagnostics, Gmbh) and ii) and the manual QIAamp DSP DNA Blood Mini kit (Qiagen, Gmbh). All swabs were tested in qPCR to determine DNA concentration and DNA quality (Quantifiler trio DNA Quantification Kit, Life Technologies).
Results: The DNA concentration ranged from 0.03 to 63 ng/µl with 66 samples (69.5%) lower than the 10 ng/µl required for genotyping protocol. The main buccal swab conditioning method was dry cotton (D) (n = 61) and others were gel matrix (G) (n = 12) and medium preserving virus or bacteria (M) (n = 16). Unfortunately, in 6 cases we did not get information about sample conditioning. Interestingly, HPA genotyping results were 100% concordant. In 2 cases, the results were unconclusive as DNA was degraded in one sample (2 LS results, DNA concentration = 1.15 ng/µl) and DNA impurities were present in the other (xB results, DNA concentration at 2.05 ng/µl). We also found that DNA concentration varied according to swab conditioning method (mean ± SD in ng/µl): D = 9.7 ± 12.6 vs G 4.7 ± 4.5 vs M 17.9 ± 20.4. Moreover, the 2 unconclusive results were collected by G method.
Summary/Conclusions: These results suggest that buccal swabs DNA concentration is related to conditioning and that the gel method provides the less accurate results. Therefore, we strongly recommend physicians not to use the gel matrix. Collecting buccal swabs is an easy and non-invasive way to genotype for platelet antigens. It will be indicated in NAIT management and pre-term neonates. However, in case of HPA genotyping failure, an EDTA blood sample must be sent in a second intention.

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SUCCESSFUL MANAGEMENT OF A HYDROPIC FOETUS WITH SEVERE ANAEMIA AND THROMBOCYTOPENIA CAUSED BY ANTI-CD36 ANTIBODY

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Background: Fetal and neonatal alloimmune thrombocytopenia (FNAIT) is one of the most common causes of severe thrombocytopenia and intracranial haemorrhage (ICH) in fetuses and term newborns. It is caused by maternal platelet antibodies (abs) against antigens expressed on fetal platelets inherited from the father but lacking in the mother. Anti-CD36 represents the most frequent platelet ab found in FNAIT cases among Asians. However, little is known about the treatment of anti-CD36 mediated FNAIT.
Aims: Analyze and manage a hydropic fetus with severe anemia and thrombocytopenia caused by anti-CD36 antibody.
Methods: Anti-CD36 abs containing in maternal serum and in the umbilical cord were tested by PAKPLUS, and the CD36 expression on monocytes and platelets were measured by flow cytometry. The CD36 gene was also analyzed by sequencing.
Results: A Chinese male fetus was diagnosed with severe FNAIT with ascites, pericardial effusions, cardiomegaly, anaemia (haemoglobin, 4.8 g/dl) and thrombocytopenia (platelet count 16 G/l) at 27 weeks of gestational age. His mother had a history of several intrauterine fetal demise and/or hydrops; five times in the last 7 years. Our immunological analysis showed total absence of CD36 on maternal platelets and monocytes, caused by two common nucleotide deletions AC at positions 329–330 of the CD36 gene. Anti-CD36 and anti-HLA class I abs could be detected in the maternal serum. However, only anti-CD36 abs was found in the fetal blood sample, indicating that anti-CD36 is responsible for this severe FNAIT case. Serial intrauterine transfusions with red blood cells (RBC) and platelets from CD36*null* donors were performed. The newborn (body weight, 2.250 g; Apgar scores 10) was delivered vaginally at 32 weeks of gestation with normal haemoglobin (18.6 g/dl), but low platelet count (48 G/l). Subsequent ultrasound of the foetus showed a total resolution of effusion, hydrops and oedema. The platelet count rose to 121 G/l on day 2. The newborn was discharged from the hospital and no neurologic abnormality was observed until today.
Summary/Conclusions: This report demonstrates that intrauterine transfusions with compatible CD36*null* RBC and platelets are useful to prevent the deleterious clinical effects of anti-CD36 mediated severe FNAIT.

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SEVERE NEONATAL THROMBOCYTOPENIA DUE TO AN ANTI-GROUP A FETO-MATERNAL ALLO-IMMUNIZATION: A CASE REPORT

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Background: Fetal or neonatal alloimmune thrombocytopenia (FNAIT) results form a maternal alloimmunization against fetal platelet (PLT) antigens. To date, 35 human PLT antigens (HPAs) have been described.
Aims: We present the case of a 29 years old French woman who gave birth to her first baby at 38 weeks of gestation (male, 3,520 g). Investigations were performed due to a suspicion of maternofetal infection at 24 h of life. No infection was detected, but a moderate anemia and a severe thrombocytopenia were observed.
Methods: Direct antiglobulin test, red cell antigen typing, irregular antibody screening and identification were done by polyspecific MISS Coombs Gel card and standard methods. Anti-platelet antibody screening, identification and cross-match with the father's platelets were performed with the MAIPA method. HPA genotypings were performed by sequencing.
Results: Maternal anti-group A antibodies were clearly identified on newborn red blood cells. Platelet immunology investigations did not reveal any platelet feto-maternal incompatibility. The cross-match of maternal serum against father's platelets was positive in MAIPA due to maternal anti blood group A antibodies. After adsorption of the maternal serum on father's RBC, the MAIPA cross-match of the maternal serum on father's platelets was negative, thus demonstrating that anti-group A antibody was responsible of the positivity.

Summary/Conclusions: This case clearly demonstrates that anti blood group A antibodies can be responsible of severe neonatal thrombocytopenia.

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FNAIT DUE TO ANTI-HPA-3A ANTIBODIES, COMPLICATED BY THE LATE DEVELOPMENT OF IRREGULAR BLOOD GROUP ANTIBODIES

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Background: To date, 35 human platelet antigen (HPA) systems have been identified. Antibodies against HPA have been involved in fetal and neonatal alloimmune thrombocytopenia (FNAIT), posttransfusion purpura (PTP) and platelet refractoriness. A single nucleotide polymorphism resulting in the substitution of the amino acid isoleucine to serine at position 843 on GPIIb, determines the HPA-3a/3b status. While anti-HPA-1a accounts for 79% of confirmed cases of FNAIT, HPA-3a antibodies represents only 2%.

Irregular blood group antibodies against antigens in the Kidd and MNS systems are involved in hemolytic disease of the newborn and hemolytic transfusion reactions.

Aims: NA.

Methods: NA.

Results: A 33-year-old gravida 5, para 3 with a previous history of FNAIT due to anti-HPA-3a was referred to our hospital at gestational week (GW) 28 for follow up. She was typed as O RhD+, with a negative antibody screening in GW 12. No HLA-antibodies were detected. We identified eight HPA-3bb blood group O donors, but only one met the criteria for donation 2 days prior to scheduled C-section at GW 38. Four days before C-section, the donor became sick, and we had to harvest platelet from an A RhD+ HPA-3bb donor. The patient's IgG anti-A-titer was 16. We also reserved two O RhD+ HPA-3bb erythrocyte concentrates in order to prevent PTP in case the patient would need blood transfusion. However, anti-Jkb and anti-S were detected in her plasma the day before C-section. Both of the HPA-3bb erythrocyte concentrates were positive for either Jkb and/or S, thus no longer compatible. No O, Jkb-, S- and HPA-3bb erythrocyte concentrates were retrieved. During C-section the total blood loss was 800 ml. The postoperative Hb was 88 g/l and she was not transfused. The newborn, a boy, presented with a platelet count of 20×10^9/l, but without any petechiae/ecchymosis or other signs of bleeding. Hemoglobin was 230 g/l. He was typed as blood group O, and had a positive direct antiglobulin test. He responded well to transfusion with blood group A HPA-3bb platelets (15 ml/kg), with an increase in platelet count to 134×10^9/l. Platelet count decreased within 48 h to 57×10^9/l, and a dose of Octagam® 0.5 g/kg was administered. No further treatment was needed.

Summary/Conclusions: Here we describe a case of anti-HPA-3a related FNAIT. A single transfusion with HPA-3bb platelets and IVIG given to the newborn provided adequate clinical response. However, due to the restricted number of available HPA-3bb donors, we had to harvest platelets with a major ABO mismatch. Moreover, due to development of anti-Jkb and anti-S by the mother late in the pregnancy, we were unable to reveal any compatible erythrocyte concentrates from HPA-3bb donors. Hence the mother would be at risk of having PTP in case of transfusion.

Little is known about why some patients are more prone to alloimmunization (responders) after transfusion or pregnancy. Even less is known if patients with platelet alloantibodies are at increased risk of developing blood group antibodies. When HPA alloantibodies are involved, especially others than HPA-1a, close monitoring by antibody screening with regard to blood group antibodies might be necessary.

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LEUKOCYTE ANTIBODIES IN PREGNANT WOMEN WITH RED BLOOD CELL ALLOIMMUNIZATION

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Background: Leukocyte antibodies against human leukocyte antigens (HLA) class I, class II and human neutrophil antigens (HNA) are formed from exposure to these antigens by transfusion, gestation or transplant, and are associated with transfusion-related acute lung injury (TRALI) and neonatal alloimmune neutropenia (NAN). Besides the antibody prevalence, also risk factors for acquisition of leukocyte antibodies are yet to be determined and present great importance for the prevention of severe and fatal transfusion reactions.

Aims: To compare the prevalence of leukocyte antibodies in pregnant women with red blood cells (RBC) alloimmunization and in multiparous blood donors without RBC alloimmunization.

Methods: We analyzed 141 blood samples from pregnant women with RBC alloimmunization and no transfusion history. In this cohort, the investigation and identification of RBC antibodies were performed by the DG Gel-Card technique (Grifols-Spain). For the control group, 467 multiparous blood donors (02 or more pregnancies) without RBC alloimmunization were included. The techniques used for the investigation and identification of leukocyte antibodies were: (i) granulocyte agglutination test (GAT), (ii) white cell immunofluorescence test (Flow-WIFT), both tests using a panel of neutrophils obtained from three donors with HNA genotyping, and (iii) bead-based assay – LABScreen Multi (LSM) (One Lambda), capable of detecting antibodies against HNA-1a, -1b, -1c, -2, -3a, -3b, -4a, -5a, -5b and HLA class I and II antigens. The identified HNA antibodies were confirmed by genotyping of the corresponding antigen.

Results: In the cohort of alloimmunized pregnant women, we found 175 RBC antibodies: anti-D (32.4%), anti-Lea (21.7%), anti-C (9.1%), anti-E (8.5%), and other antibodies (28.3%). Regarding the gestational history, 74 (52.4%) women had two or more pregnancies. We identified 70/141 (49.6%) samples with anti-HLA antibodies and 10/141 (7.0%) samples with anti-HNA antibodies with the following specificities: 4/10 anti-HNA-1a, 2/10 anti-FCγRIIIb and 4/10 anti-HNA-3b. In the control group, we found 199/467 (42.6%) samples with anti-HLA and 23/467 (4.9%) samples with anti-HNA: 7/23 anti-HNA-1a, 01/23 anti-HNA-1c, 03/23 anti-HNA-2, 02/23 anti-HNA-3a, anti-HNA-3b, 03/23 anti-HNA-5a and 02/23 anti-HNA-5b. Statistical analysis was performed with paired samples, and only multiparous women in both groups were selected. 9/74 (12.1%) multiparous pregnant women with RBC and HNA alloimmunization were compared with 23/467 (4.9%) multiparous blood donors with HNA alloimmunization only, and a significant difference was observed between the two groups [P = 0.02, OR = 2.673 (95% CI, 1.185–6.030)].

Summary/Conclusions: RBC alloimmunization was shown to be a significant risk factor (P = 0.02, OR = 2.7) for the development of antibodies against other blood cells, such as leukocytes and granulocytes, considered to be fundamental in the pathogenesis of TRALI. A high frequency of HNA alloimmunization (12.1%) was observed in RBC alloimmunized group vs 4.9% in the control group, suggesting that they are better immune responders and that they react strongly to allogeneic exposure.

P-560

A CASE OF SEVERE FETAL ANEMIA DUE TO ANTI-KELL THAT COULD NOT BE DETECTED BY THE WEEKLY ASSESSMENT OF MCA-PSV

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Background: Hemolytic disease of the fetus and the newborn (HDFN) caused by anti-Kell antibodies is a rare but serious disease that can cause hydrops fetalis and intrauterine death. The monitoring of fetal anemia is thus important to prevent such outcome via an appropriate and timely intervention. The Doppler measurement of fetal middle cerebral artery peak systolic velocity (MCA-PSV) is at this time the best non-invasive test to predict fetal anemia in alloimmunized pregnancies.

Aims: The recommended cut-off value of MCA-PSV as an indication of fetal blood sampling (FBS) and the recommended interval between the repeated measurements are the same for all antibodies whatever the specificity. The aim is to show that these recommendations should be adapted for Kell-alloimmunized pregnancies as anti-Kell HDFN is a different disease from that due to other alloantibodies.

Methods: We describe a case of severe fetal anemia due to anti-Kell antibodies that could not be detected by the weekly assessment of MCA-PSV.

Results: An anti-Kell antibody at a titer of 512 was detected in the serum of a 31-year-old pregnant woman at 6 + 4 weeks of gestation. During the 27th week of gestation, the patient presented to the emergency department because she couldn't detect fetal movement. The Doppler measurement of MCA-PSV performed 2 days before indicated mild anemia (corresponding to an hemoglobin (Hb) concentration between 8.1 and 10.5 g/dl). In line with the recommendation, no intervention was performed and no signs of hydrops were seen at ultrasonography. The new measurement indicated severe anemia (corresponding to an Hb concentration under 6.9 g/

dl). A FBS was performed to confirm severe anemia and the fetal Hb concentration was 2.4 g/dl. The fetus had signs of hydrops at ultrasonography. The mother received a total of 4 fetal blood transfusions. The delivery was timed at 37 weeks of gestation and a baby boy was born with a mild jaundice.
Summary/Conclusions: It is really important to detect severe fetal anemia prior to the onset of hydrops since it might improve the outcome for the fetus. In this case of Kell-alloimmunized pregnancy, the kinetic of development of fetal anemia was really quick and the weekly assessment of MCA-PSV using the recommended cutoff value as an indication of FBS failed to detect fetal anemia before fetal hydrops develops. The optimal MCA-PSV cutoff value as an indication of FBS and/or the interval between repeated MCA-PSV measurements need to be adapted in future studies for pregnancies complicated by anti-Kell antibody as the mechanism of fetal anemia is different from other kind of alloimmunized pregnancies. Education of all Kell sensitized pregnant women is also important since that was the absence of fetal movement detected by the mother that probably saved the fetus.

P-561

HEMOLYTIC DISEASE OF THE FETUS AND NEWBORN CAUSED BY ANTI-BEA

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Background: HDFN caused by antibodies to low prevalence antigens are reported rarely. In such cases screening for the presence of antibodies during pregnancy is not informative because the reagent red cell panels used seldom contain RBCs expressing low frequency antigens. The occurrence of the disease in the neonate is most often a surprise for obstetricians. We report here a case of mild HDFN caused by antibodies to the rare low prevalence Rh antigen, Be^a (RH36).
Aims: Case report: A 33-year old Polish woman who presented with a negative antibody screen during her current (second) and previous pregnancy, delivered at term a baby girl with jaundice. At birth Eryt: 3.63 × 1,012/l, Hb: 8.2 mmol/l, Ht: 0.375/l, reticulocytes: 98%, bilirubin: 228.7 μmol/l. The baby was treated with IVIg (KIOVIG) and phototherapy. After delivery maternal samples were examined for the presence of alloantibodies and her serum was found to be reactive only with cells from the baby's father and therefore an antibody to a low prevalence antigen was suspected. Further investigation revealed the presence of anti-Be^a. The child was discharged from the hospital 4 days later. We were informed that a month later at a routine visit, Hb level was 11.0 mmol/l.
Methods: DAT was performed by gel method (Bio-Rad). Eluates were prepared by acid elution method (DiaCidel Bio-Rad). Serological investigation was performed by standard LISS tube and Bio-Rad IAT techniques. Genomic DNA was extracted from whole blood and subjected to sequencing of exons 1–10 of the *RHCE* gene
Results: The newborn's cells were found to be DAT positive with anti-IgG; an eluate prepared from her cells reacted with the father RBCs. The newborn's plasma was weakly reactive with the father's cells. Serum of the mother reacted with cells from the father, her newborn daughter and with Be(a+) RBCs, all other cells tested were compatible. The father's and newborn's cells were found to be ccdee Be(a+); the mother's cells were found to be ccdee Be(a−). *RHCE* sequencing revealed the father and newborn, to have the heterozygous mutation 662C>G in exon 5, resulting in a Proline to Arginine change at position 221 of the RhCcEe protein. The expected polymorphisms in exon 2 and 5 that are associated with the RHCE*ce/ce genotype were also identified. These results are characteristic of the *RHCE*ce/ceBE* genotype.
Summary/Conclusions: We have presented a case of anti-Be^a which caused HDFN. This is only the fourth case of anti-Be^a to be described. The previous three cases also implicated anti-Be^a as the cause of HDFN in the second pregnancy. This case provides further evidence regarding the clinical significance of this very rare antibody in pregnancy.

P-562

RED CELL IMMUNIZATION IN PREGNANT WOMEN AT THE TRANSFUSION CENTRE, GENERAL HOSPITAL CELJE IN A 7 YEAR PERIOD FROM 2010 TO 2016

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Background: Pregnant women with alloantibodies were serologically monitored with the aim of treating and preventing haemolytic disease of the foetus and newborn.
Aims: We wanted to analyse frequency of clinically significant antibodies occurring in pregnant women treated in our hospital in period from 2010 to 2016, especially anti-D and anti-Kell antibodies which can cause severe haemolytic disease of the foetus and newborn.
Methods: The new immunizations in pregnancies in a 7 year period from 2010 to 2016 at the General hospital Celje are presented. The data were collected from the Datec information system, hand-held files for pregnant women and annual reports of the General hospital Celje.
Results: In a 7 year period, 19,822 pregnant women were treated; among them 123 (0.6%) were immunized to red cell antigens. The majority of clinically significant alloimmunizations were found in the Rh system: 34 (27.4%), among them, 14 had anti-E, 8 anti-Cw, 5 anti-C,D, 4 anti-D,2 anti-C and 1 had anti-e antibodies. Two pregnant women with anti-D antibodies came from a country where antenatal RhD prophylaxis is not implemented. The third pregnant woman developed anti-D antibodies during her third pregnancy. We did not obtain reliable information about RhD prophylaxis in her previous pregnancies. The fourth pregnant woman developed anti-D antibodies in her second pregnancy before 27th week. The Commbs screening test in the beginning of her second pregnancy was negative. In the Kell system 7 (5.6%) immunizations were found, among them there were 5 anti-Kell and 2 anti-Kp (a) antibodies. Three pregnant women developed anti-Kell antibodies after receiving transfusion of Kell positive blood more than 10 years ago. One pregnant woman gave birth to Kell positive child in previous pregnancy. In one case source of sensibilization was not found. In Kidd system 3 (2.4%) antibodies were found and 2 (1.6%) antibodies in Duffy system. In the MNS and Lewis systems 42 (33.9%) antibodies were found, these are mostly naturally occurring antibodies. In 35 (28.2%) immunizations antibody specificity could not be determined. One pregnant woman had two antibodies.
Summary/Conclusions: In Slovenia post-partum RhD prophylaxis is performed since 1970 and antenatal RhD prophylaxis since 1992. Despite that 4 immunizations to RhD antigen in pregnant women were detected in period from 2010 to 2016. Two of them did not receive antenatal prophylaxis. However anti-D antibodies represent 3.2% of all antibodies in pregnant women in period from 2010 to 2016 in analysed period comparison to 20% in period from 2005 to 2009 which is due to consistent performance of antenatal and post partum RhD prophylaxis. Anti-Kell antibodies in pregnant women represented 4.0% of all antibodies in analysed period comparing with 18% in period from 2005 to 2009. Decrease in percentage of anti-Kell antibodies detected is due to preparation of Kell compatible blood for transfusion which is performed since 2007.

P-563

Abstract has been withdrawn.

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A STUDY OF ALLOIMMUNIZATION IN PREGNANT WOMEN FROM THE DEMOCRATIC REPUBLIC OF CONGO, AFRICA

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Background: Screening for the presence of maternal antibodies is standard procedure in developed countries and of great importance for the prevention of hemolytic disease of the fetus and newborn (HDFN). In Sweden antibodies against the D-antigen is still considered to be associated with most of the severe cases of HDFN. The presence of red cell antibodies is estimated to occur in approximately 1% of all pregnancies in Sweden. In the African population the D-antigen is less common and

scientific reports regarding the incidence of red cell antibodies during pregnancy are few.
Aims: The aim of the present study was to screen for antibodies among pregnant women at the Panzi Hospital, Bukavu situated in eastern part of Congo.
Methods: Samples were collected from pregnant women at the Panzi Hospital. The ABO and RhD blood group were determined with tube technique. The plasma was frozen and screening for antibodies were performed at the Sahlgrenska University Hospital using Bio-Rad gelcards and indirect antiglobulin technique (IAT). The screening panel consisted of three cells. Positive samples were further investigated using IAT a gel standard panel. The ethical committee at the Panzi Hospital has approved the study design.
Results: Samples were drawn from 473 pregnant women, gestational week 22–38 with median week 27. Blood group O RhD positive were the most common blood group (41.2%) and in total 28 women were RhD negative (5.9%). When screening for antibodies, 20 samples were found to have weak positive reactions and two samples had strong positive reactions. In the following antibody identification most samples were found to be negative or indeterminate. The two strong positive reactions correlated with two clinical significant antibodies, i.e. anti-D and anti-e. Furthermore an anti-Lea were identified. Overall the frequency of antibodies were found to be 0.6%.
Summary/Conclusions: The frequency of RhD negative individuals in Congo has to our knowledge not previously been reported. The frequency was unexpectedly high compared to previous studies from Africa, in literature often reported to be 1–2%. The speculation is that this could be due to population differences or due to weak or variant RhD. Further studies is needed with additional techniques. Maternal antibodies were found in a somewhat lower frequency compared to the Swedish context. The significance of this finding is not clear. This study is a small pilot project with the long term ambition to improve the health care for pregnant women including prenatal screening for red cell antibodies in Congo.

P-565

SCREEN AND ANTIBODY IDENTIFICATION, SOLID PHASE VS CATN IN PRE-NATAL SAMPLES

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Background: The Capture R Pool solid phase adherence test has been widely used for screening patient and pre-natal samples for alloantibodies against red cells. Each sample is tested against the red cell membranes of a pool of donors selected so the combination of their antigen profiles will detect clinically significant antibodies.
Aims: The aim of this study was to determine how effective and sensitive was pool screen method when using pre-natal samples. During a period of 5 months, 1,052 samples were performed in Pool screen (solid phase) and in 2 Cell screen (solid phase) for comparison and correlation purposes, was also performed 3 cell screen on Column Agglutination Technique (CAT) from Grifols. Results were compared and an identification panel from both techniques was then performed (solid phase and CAT) to determine the presence of antibodies.
Methods: Pre-Natal samples were tested on Neo instrument (Immucor) using Pool screen (solid phase, Capture R Pool, Immucor). Samples reacting positive in those tests were then reflexed to 2 Cell screen (solid phase, Capture R I+II, Immucor) and 3 cell screen (Serascreen, Grifols). If the results were positively consistent, further investigation was performed using identification panels, solid phase (Capture R ID, Immucor) in Neo Platform and CAT panel (IDENTISERA DIANE P, Grifols) in Erytra, to establish which antibodies were present.
Results: Out of 1,052 samples 46 were positive in Screen Pool. Of the 46 positive screens 36 were concordant between solid phase method and CAT. Only 10 samples were discrepant between the 2 methods. Two false positive samples for solid Phase and 4 false negative for CAT of which, two Coombs positive samples, 1 Anti-Cw and 1 Anti-D (all detected by solid Phase). Two Anti-M were identified by CAT and positive unclear by solid Phase.
Summary/Conclusions: The vast majority of identified Anti-Ds were due to Gamma globulin prophylaxis. The Capture pool screen method and Capture 2 Cell Screen (solid phase) present an important level of sensitivity for the detection of antibodies, even when dealing with interferences produced by a direct positive Coombs for example. Anti-M was not clearly identified in the NEO because this antibody needs incubation at 22°C for its correct identification (cold antibody), nevertheless the NEO detected the presence of the antibody in the Screen. An Anti-D NOT secondary due to prophylaxis was detected first in the NEO and the screen was negative in CAT, the NEO detected it showing small reaction intensity (probably due to low titre) but towards a correct identification, 2 weeks later it was positive in gel. And most importantly, if the NEO had not been positive for the Screen, it would not have been possible to diagnose the Anti-Cw, since the Screen in CAT was negative. The identification of this antibody was correct, reason why its sensitivity is important to be able to identify antibodies directed against antigen of low incidence. Although further studies are needed, that comprises other antibodies from different systems such as Duffy, Kell, Kidd. We can claim that Capture (Solid Phase) is very useful method in the screening of pre-natal samples.

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Abstract has been withdrawn.

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RHD ANTIBODIES IN PREGNANT WOMEN IN MULTI-ETHNIC SURINAME: THE OBSERVATIONAL RHESUN STUDY

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Background: Maternal antibodies against the RhD antigen is the most common cause of severe hemolytic disease of the fetus and newborn (HDFN). In high-income countries, the risk of RhD immunization has reduced by routine antenatal and postpartum administration of anti-D immunoglobulin from 13% to below 0.5 percent. In less-resourced countries, such as Suriname, red blood cell antibody screening during pregnancy and prophylactic anti-D immunoglobulin administration are not routine. Accurate data on RhD immunization risk is not available.
Aims: In the RheSuN (Rhesus Surinamese Neonates) study, the prevalence and the hemolytic potential of maternal RhD antibodies were investigated.
Methods: A multi-center cross-sectional study in four major hospitals in Paramaribo, Suriname, covering 90% of about 10,000 newborns yearly in Suriname. Included were RhD-negative pregnant women and/or their newborns of various ethnicities, seeking routine prenatal.
Results: RhD antibodies were detected in 19 of 214 RhD-negative pregnancies (8.9%; 95% CI 5.1–12.7%); in 2.0% of primigravid and 11.7% of multigravid women. The DAT was positive in 11 of 13 tested RhD-positive newborns. Treatment for RhD antibody induced hemolytic disease of the fetus and newborn (HDFN) with exchange transfusions and/or phototherapy was performed in four DAT positive newborns.
Summary/Conclusions: RhD immunization risk and HDFN treatment frequency in Suriname women are comparable to the pre anti-D prophylaxis era in high income countries. Free of charge routine RBC antibody screening and prophylactic anti-D immunoglobulin administration for women at risk for RhD antibody formation as part of standard of ante- and postnatal care is recommended.

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ANTI-D QUANTIFICATION IN MONITORING IMMUNIZATION DURING PREGNANCY

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Background: The monitoring protocols of anti-D immunized pregnancies differ between centers and countries. In most centers anti-D titers are followed and

interventions are initiated at high titers or if the titers rise quickly. In some centers anti-D quantification is advocated, in addition to titers.

Aims: The aim of this retrospective study was to analyze how anti-D quantification correlates to anti-D titers and if it adds important predictive information to the outcome of the pregnancy.

Methods: During the period October 2013 to December 2016 immunized women with anti-D titer ≥128 at any time during pregnancy, were included. At titer ≥128, anti-D quantification was performed and middle cerebral artery (MCA) peak systolic velocity (PSV) was measured. Treatment with intrauterine transfusions (IUT) was administered at MCA PSV > 1.5 MoM (Multiple of the Median) for gestational length, according to protocols. Titers were performed with gel card technology (Bio-Rad, Germany) and anti-D quantification was done by flow cytometry. The laboratory participates in proficiency testing schemes (EQUALIS, SE, NIBSC, UK). Clinical and laboratory information were collected from the medical records and laboratory information systems.

Results: In 69 pregnancies in 62 women 478 titers and 295 quantifications were performed. The median titer was 256 [range 0–128,000] and median quantification value was 10 IU/ml [range 0.3–6,024]. Titers correlated to quantification ($R = 0.78$), but there were large variations at each titer value, e.g.: 128 (1.2–12 IU/ml), 256 (1.7–24 IU/ml), 512 (1.7–42 IU/ml), 1,024 (6.3–113 IU/ml), 2,048 (10–124 IU/ml). IUT was initiated in 32 pregnancies (1–8 IUT/pregnancy) at titer 64–8,192 and concentrations 3.4–105 IU/ml, mainly based on MCA PSV.

Vaginal delivery was done in 28 and cesarean section in 29 pregnancies (gestational week 36 median, range 32–40) all with live newborns. Five pregnancies resulted in miscarriage, two in termination due to severe growth retardation and in five the outcome were unknown. 49 of the newborns had hyperbilirubinemia and were treated with phototherapy (46) and exchange or top-up transfusions (31).

Summary/Conclusions: Despite a standardized method for anti-D quantification controlled by internal and external controls and standard curves, titers and anti-D quantification varies in relation to each other, to MCA PSV and to the outcome of the pregnancy in non-predictable ways. These data will be analyzed more in detail and on individual basis to better understand and optimize the monitoring of anti-D immunized pregnancies.

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ANTI-D QUANTITATION BY CONTINUOUS FLOW ANALYSIS: COMPARATIVE STUDY OF TWO DIFFERENT METHODS ON TECHNICON/ALLIANCE INSTRUMENTS AND ON WHITE HORSE SCIENTIFIC SYSTEMS

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Background: Anti-D quantitation by continuous flow analysis (CFA) on Autoanalyser is routinely used for decades in the laboratory of the French National Center for Perinatal Hemobiology (CNHRP) for the management of allo-immunized pregnancies. This assay is performed with a method using polyvinylpyrrolidone (PVP) as "rouleaux" inducing agent on Technicon Autoanalysers and MS Alliance Evolution III systems. In 2014, the laboratory was equipped with Astoria Pacific systems (White Horse Scientific) to replace the Technicon Autoanalysers. A new method using methylcellulose instead of PVP has been developed on these systems.

Aims: The aim of this work was to compare the performance of the two methods.

Methods: Red blood cells of R^1R^2 phenotype were bromelinized prior to the test (« 2-stages »). In a continuous flow, pre-diluted samples of patients sera are added to the cells in the presence of "rouleaux" inducing agent. After incubation coils, dispersion of rouleaux is achieved by addition of isotonic saline in the circuit. Only immunological formed agglutinates remain. Agglutinated cells are then removed by decantation. Remaining red blood cells are lysed by Triton and supernatant content of hemoglobin is measured with a spectrophotometer at 550 nm. The International anti-D Standard (01/572) is used to calibrate a working secondary standard that allows the expression of results in IU/ml. A low and a high level of internal quality controls (IQC) and an international standard sample were used to determine the intraassay and interassay imprecisions. Accuracy was determined based on *z*-score values obtained after retrospective dosage of external quality controls from the UK NHSBT AQQAS quality assurance scheme. Comparison of the results obtained from the same clinical samples with both methods was carried out with Deming regression and measures of Bland-Altman bias.

Results: For methylcellulose and PVP methods, the intraassay imprecision was determined on the international standard level and shows comparable means (6 vs 6.4 IU/ml) and coefficients of variation (CV) (6% vs 7%). The interassay imprecision was calculated on the low and high level of IQC and shows comparable means (1.2 and 5.3 vs 1.4 and 5.2 IU/ml) and CV (15% and 17% vs 18% and 17%). The accuracy was determined on 19 frozen AQQAS samples and the means of the calculated *z*-score were respectively 1 and 1.5. The Deming regression equation obtained on 75 clinical samples was $Y = 0.83X + 43$ ($r = 0.990$). The mean bland-Altman bias was 1.05 with 3 diverging points.

Summary/Conclusions: Both methods show comparable results in terms of analytical performance and a good correlation coefficient. The threshold concentration of 5 IU of anti-D/ml used to determine when clinicians should monitor the pregnancy by ultrasonography because of a risk of fetal anemia remains the same for both methods. The high scattering of Bland Altman ratio is probably due to technical differences ("rouleaux" inducing agent used, auto-analyzer specificities) and to the fact that methylcellulose method works in a larger excess of antigen with linear hemagglutination curves instead of logarithmic ones. Thus, the biological follow-up of each anti-D allo-immunized pregnancy has to be done always with the same method.

P-571

CLINICAL INPUT OF ANTI-D QUANTITATION BY CONTINUOUS FLOW ANALYSIS ON AUTOANALYSER IN THE MANAGEMENT OF SEVERE ANTI-D MATERNAL ALLO-IMMUNIZATION

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Background: Beside titration by indirect antiglobulin test most widely used, anti-D quantitation by continuous flow analysis (CFA) may be performed to assess maternal immunization severity. Only few studies have related its interest in the management of pregnancies complicated by anti-D immunization. To reduce the severity of the hemolytic disease of the fetus and the newborn (HDFN) in pregnancies at higher risk of early intrauterine fetal transfusion (IUT), a preventive high-dose intravenous immunoglobulin (IVIG) therapy may be introduced. The effect of IVIG treatment on maternal anti-D CFA concentrations has not yet been established.

Aims: The aim of our study was firstly to demonstrate the relevance of anti-D CFA quantitation for the management of pregnancies complicated by anti-D immunization and secondarily to assess its added value for the biological follow-up of patients treated with IVIG treatment.

Methods: A retrospective study of 86 severe anti-D immunized pregnancies (anti-D titer > ou = 16) followed at the Trousseau hospital between 2013 and 2014 was conducted. Concentrations of maternal anti-D were measured by CFA 2-stages method (2SM) (total amount of anti-D) and 1-stage method (1SM) (high affinity IgG1 anti-D). Simultaneously, titrations were performed. These biological data were compared to the severity of the antenatal HDFN (need of IUT, gestational age of the first IUT, fetal cord hemoglobin concentration before the first IUT). For 6 severely anti-D allo-immunized pregnant women treated with IVIG and followed at the Trousseau Hospital between 2013 and 2015, the kinetic of anti-D CFA concentrations was analyzed since IVIG introduction and until the first IUT.

Results: The value of 5 IU of anti-D/ml in maternal sera is validated as a threshold to trigger ultrasonographical and doppler fetal surveillance in order to detect fetal anemia. For pregnancies requiring IUT (n = 36), maternal 1SM anti-D concentration correlates significantly with the severity and the precocity of the fetal anemia. In 5/6 pregnancies with IVIG treatment, anti-D 1SM and 2 SM concentrations significantly decrease after IVIG introduction, suggesting that one of the mechanism of action of this treatment to delay the outcome of the fetal anemia is the reduction of the maternal antibody load.

Summary/Conclusions: Altogether our results underline the interest of anti-D quantitation by CFA to optimize the management of severe anti-D allo-immunized pregnancies.

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QUANTIFICATION OF FETO-MATERNAL HEMORRHAGE: VALIDATION OF AN INDIGENOUS METHODOLOGY IN RESOURCE POOR SETTING

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Background: RhD sensitization in RhD-negative mothers continues to be a major health problem in developing nations like India. Inadequate immunoprophylaxis is the main reason. Easy-to-perform tests are required to quantify feto-maternal hemorrhage (FMH) and thereby calculate the correct anti-D dose.
Aims: Identify a cheap and easy-to-perform technique for quantifying FMH, in a resource-poor setting.
Methods: We evaluated three quantitative and one qualitative techniques of estimating FMH. The Kleihauer-Betke test (KBT) and two flow cytometry (FC) techniques such as the indirect immunofluorescence technique (IIFT) and direct immunofluorescence technique (DIFT) were the quantitative ones, while gel agglutination technique (GAT) was the qualitative one. Stock solutions of both RhD-positive cells and RhD-negative cells containing approximately equal red cell counts were prepared using a calibrated automated cell counter. 7 serial dilutions of RhD-positive cord cells in RhD-negative adult cells were prepared to yield final D-positive cell concentrations of 0.06%, 0.12%, 0.25%, 0.50%, 0.75%, 1% and 2%. All samples were tested by KBT, DIFT, IIFT and GAT. For GAT, serial dilutions of monoclonal IgG anti-D were incubated with each dilution of RBC mixtures and supernatant was tested against "O" group RBC using anti-IgG gel cards.
Results: KBT could detect FMH in 80% samples when concentration of D positive cells was 0.06%. KBT could quantify fetal cells in all samples tested; however fetal cells were underestimated when expected cell concentration was ≥0.75%. By FC analysis, a higher accuracy was observed by DIFT than IIFT. However an underestimation was observed particularly by IIFT when expected RhD+ cell concentration was ≥1.0%. In all samples analyzed by FC, both IIFT and DIFT could accurately detect and quantify FMH. There was good correlation between FC and KBT. Each method showed good correlation between expected and measured concentrations of D+ cells. At a concentration of ≥0.25%, all techniques were comparable and equally sensitive. FC was found to be the most sensitive technique and could detect RhD positive/HbF positive events accurately even at a cell concentration of 0.06%. The efficiency of GAT was more appreciated when fetal cell contamination was ≥ 0.25%. It corresponds to >5 ml of FMH – the clinically significant cut-off of FMH. We developed an indigenous calibration curve using known serial concentration of fetal RhD positive and maternal RhD negative RBCs. This curve determines the volume of FMH by depicting the agglutination reaction against a particular anti-D dilution and converts the qualitative test into a semi-quantitative type.
Summary/Conclusions: FC was found to be the most sensitive of all techniques. But very few laboratories in the developing nations can afford such costly device. KBT has been the gold standard for quantification of FMH. However, the technique requires considerable expertise. Blood centers using gel cards for red cell serology can easily adapt GAT for FMH estimation without any extra cost. We established a "best fit" calibration curve. This curve can determine the volume of FMH by depicting the agglutination reaction (≥1+) against a particular anti-D dilution. Other centres can use this calibration curve and estimate FMH from maternal antibody titre.

P-573

THE METHOD VALIDATION OF LABORATORY MEASUREMENT OF FETOMATERNAL HEMORRHAGE BY FLOW CYTOMETRY ACCORDING TO PRACTICAL GUIDELINES FROM ICSH AND ICCS

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Background: Massive fetomaternal hemorrhages (FMH) can lead to life threatening anemia in the fetus and the newborn child. The pathogenetic background for the hemorrhages is placental malfunction, trauma, or choriocarcinoma. Most clinical laboratories in Taiwan perform FMH estimation with the Kleihauer-Betke test (KBT), while the limitations of this manual microscopic visual counting method for fetal red blood cells (RBCs) are well documented, such as labor exertion, subjectivity, imprecision, and overestimation of the extent of FMH.
Aims: The specific aim of our study was to establish and verified the quantitative assay of fetal RBCs by flow cytometry in maternal blood, including evaluation of accuracy, linearity, precision, sensitivity, and reference intervals.
Methods: Quantification of FMH by flow cytometry is based on the detection of fetal red blood cells using a monoclonal anti-HbF antibody. The accuracy was checked by comparing CAP (College of American Pathologists) proficiency test samples with peer results. Method comparison between flow cytometry and KBT was performed with 20 samples. Linearity check was performed with the serial dilution of newborn blood with normal adult blood ranged from 1:10 to 1:320. Precision check was performed with two levels of control samples prepared from the mixture of newborn blood and adult blood. Sensitivity defined by limit of detection (LOD)/limit of blank (LOB) were established by performing with 5 low positive and 5 blank samples with 5 runs in 3 separate days.
Results: The accuracy checks were acceptable within the allowable range of CAP peer results and precision checks also qualified compared with the precision of the CAP samples results ranges. Limit of blank (LOB) was 0.04%. Lower limit of quantification was 0.08% proved to equal to LOD. Reference intervals for parturient-delivered full-term babies are 0–0.18% and 0–0.19% respectively by flow cytometry and KBT.
Summary/Conclusions: In conclusion, we set up and verified the quantification of fetal RBCs in maternal blood by acid elution test and flow cytometry-based method.

P-574

A SIMPLE AND LESS TIME CONSUMING STRATEGY TO CONFIRM UNEQUIVOCALLY THE PRESENCE OF ANTI-D IN AN ANTI-(D+C) SPECIFICITY FROM THE IDENTIFICATION PANEL RESULTS

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Background: Anti-D is known to be the most clinically important antibody able to induce severe hemolytic disease of the fetus and of the newborn (HDFN) and hemolytic transfusion reactions. Due to its clinical importance, especially in the context of pregnancy, it is essential to confirm or to exclude the presence of anti-D in the sera that shows apparent anti-(D+C) specificity using easily performed protocols, which may prevent the unnecessary administration of prophylactic anti-D immunoglobulin.
Aims: We propose a new protocol in which it is not necessary to resort to techniques of adsorption and elution. It only requires the analysis of the reaction intensity obtained against R_2R_2, R_1R_1, R_0r, and r'r cells of the regular panels used for irregular antibody identification to infer the presence or absence of anti-D.
Methods: We studied 71 previously tested samples in which apparent anti-(D+ C) specificity was found. According to our protocol we consider that anti-D is present when the intensity of the reaction with R_2R_2 cells is stronger than with R_1R_1 cells. On the contrary, anti-D is absent when the intensity of the reaction with R_2R_2 is weaker than with R_1R_1. In cases of samples with the same intensity reaction with both cells, a titration was performed with R_2R_2 and R_1R_1 cells in order to find the dilution in which $R_2 > R_1$ or in which $R_2$1 could be discriminated. When $R_2 = R_1$ at all dilutions, it is necessary to perform a new titration using R_0r and r'r cells. Then, it can be concluded that anti-D is present if the reaction intensity with R_0r is stronger than the reaction intensity with r'r, and that anti-D is absent if the reaction intensity with R_0r is weaker than the reaction intensity obtained with r'r. The key point of this new approach is the precise evaluation of the different degrees of reaction obtained against the red cells with the different Rh phenotypes described.
Results: In 11 out of the 71 cases studied $R_2 > R_1$, inferring that anti-D was present. In 24 cases $R_2$1, inferring that anti-D was absent, and in the remaining 36 cases no conclusive results could be obtained because $R_2 = R_1$. After titration we were able to conclude that anti-D was present in 29 cases ($R_2 > R_1$) and absent in 5 ($R_2$1). In the 2 remaining cases $R_2 = R_1$ and an additional titration with R_0r and r'r cells was necessary showing that $R_0 > r'$ and supporting that anti-D was present. The results obtained with the 71 samples were in complete agreement with those obtained with previously reported procedures.
Summary/Conclusions: The new protocol allowed us to confirm or exclude the presence of anti-D with a simpler and less time consuming procedure, making unnecessary the use of adsorptions and/or elutions. Importantly, this method is readily accessible to all laboratories and, therefore, it reduces the need to outsource samples to reference laboratories.

P-575

RHD/HPA SIMULTANEOUS GENOTYPING FOR RESOLVING SEROLOGIC DISCREPANCIES AND CORRECTLY MANAGE PREGNANT WOMEN

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Background: RhD typing is part of antenatal testing and the correct classification of D antigen as positive, negative or variant has clinical relevance in terms of prevention of immunization. Molecular immunohaematology methods are utilised when discrepancies were encountered in patients typing, but they often give more information than required.
Aims: The aim of this study was to assess a targeted molecular test that allows to prevent the main immunohaematological diseases in pregnancy, haemolytic disease of the newborn (HDN) and foetal/neonatal alloimmune thrombocytopenia (FNAITP), resolving serological discrepancies in Rh typing for purposes of immunoprophylaxis and performing at the same time a screening program for FNAITP.
Methods: ID RHD XT (Grifols) is a qualitative, PCR-based and hybridization-based genotyping test for the simultaneous identification of multiple alleles of the RHD gene (RHD*01W.1, RHD*01W.2, RHD*01W.3, RHD*04N.01, RHD*03N.01, RHD*01N.01) and HPA-1 system using LUMINEX technology. 15 pregnant women (1 D positive, 4 D negative, 10 with Weak D or partial D serological phenotypes), previously genotype with other molecular tests (Bag-Gene PCR-SSP, RHD Bead Chip Immucor) were enrolled in this validation study, together with 24 donor samples and 13 patients (Total Number = 52).
Results: Among all studied samples, genotyping results were 100% concordant with those obtained with other molecular tests and no sample required repetition because of a failure to reach all internal QC requirements, thus giving excellent performances in terms of accuracy and robustness. As regards pregnant women samples, RHD*01W.1, RHD*01W.2 were correctly identify as well as RHD negatives (RHD*01N.01, RHD*03N.01, RHD*04N.01). Otherwise other D variants were defined as not detected. In all cases the clinical decision on treating as D positive or negative samples is clear. As regards HPA typing, no maternal sample was HPA-1a negative.
Summary/Conclusions: The use of molecular tests leads to a not only better but safer treatment of pregnant woman and to the rational use of anti-D IVIG. The use of ID RHD XT has proved to be easy to perform and targeted to specific diagnostic tool. Further tests will allow us to routinely implement this technology in our Immunohaematology laboratory.

P-576

PATERNAL RHD ZYGOSITY DETERMINATION IN IRAN

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Background: Determination of RHD zygosity is important for management of anti Rh immunoglobulin consumption in order to prevent HDFN. The RHD gene is flanked by two highly homologous DNA segments called upstream and downstream Rhesus Box. In haplotypes with an RHD deletion the fusion of the two Rhesus box generates the single-hybrid Rhezus box gene, the detection of which has been applied for RHD zygosity determination. So homozygous RhD positive individuals are negative for hybrid Rhesus box gene whilst in heterozygous a single one is detectable.
Aims: we conducted this study to investigate RHD zygosity in phenotypically RhD positive partners of women with RhD negative phenotype in Iranian population for first time.
Methods: Blood samples were harvested from 30 volunteers with mentioned criteria. RhD and RhCE phenotypes were serologically determined by anti-D, anti-C, anti-c, anti-E, and anti-e antibody. DNA was isolated from each blood sample by DNA bonding column. Hybrid Rhezus box gene was detected by PCR-SSP. Finally PCR-RFLP was performed in order to confirm PCR-SSP results.
Results: Rh phenotyping of samples revealed 26.8% DCcEe, 40% DCCee, 23.3% DCcee, 3.3% Dccee, 3.3% DccEe and 3.3% DccEE. Genotyping analysis indicated 26.6% of our samples were heterozygous and 73.4% of them possessed homozygous for RHD gene. Our results were also shown that both PCR-SSP and PCR-RFLP methods had similar efficiency in determining of RHD zygosity and no discordance were observed between these two methods.
Summary/Conclusions: We were able to determine RhD zygosity by molecular analysis of hybrid rhesus box. According to the results, both PCR-SSP and PCR-RFLP are efficient and concordant in determining of RHD zygosity.

P-577

Abstract has been withdrawn.

P-578

EXTERNAL QUALITY ASSURANCE OF NONINVASIVE FETAL RHD GENOTYPING

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Background: Alloimmunization against the D antigen in pregnant RhD negative women is preventable using anti-D immune prophylaxis. Noninvasive testing of the fetal RhD genotype allows targeted use of antenatal prophylaxis, avoiding unnecessary treatment of RhD negative women who are carrying an RhD negative fetus, and are thus at no risk of immunization. Routine fetal RhD genotyping using cell-free fetal DNA (cffDNA) has been introduced as a national service in several countries. Quality assurance of routine fetal RhD genotyping has become essential to control assay robustness and reliability. Furthermore, several laboratories require annual external quality assurance.
Aims: We conducted an international external quality assurance workshop, EQA2016, to evaluate noninvasive fetal RhD genotyping. The aim was to provide test material for laboratories to evaluate their setup for routine fetal RhD genotyping and clinical recommendations for antenatal prophylaxis.
Methods: Each laboratory tested two samples from pregnant RhD negative women. Test material was provided from one site and consisted of aliquots of pools of plasma from RhD negative women from 25 weeks of gestation. Pool 1 was positive with fetal *RHD*, and pool 2 was negative with fetal *RHD* (tested with *RHD* exons 4, 5, 7, and 10). 22 laboratories from the blood transfusion community participated. Each laboratory was instructed to provide the fetal RhD genotyping result and clinical recommendation for antenatal prophylaxis.
Results: All participating laboratories found pool 1 to be positive and gave appropriate clinical recommendations for anti-D prophylaxis. No false-negative results were observed. One false-positive and three inconclusive results were reported from the testing of Pool 2. The false-positive result for Pool 2 had Ct-values 4.5 units higher than those from the positive result for Pool 1 from this lab, suggesting either a very low level of *RHD* cffDNA, or a trace of contamination, in their Pool 2. One inconclusive result was clearly positive by exon 5 but negative by exon 7; the two other inconclusive results were weakly positive with 2/12 positive reactions in one case and 2/3 in the other but with high Ct-values of 44.4 and 46.5. The clinical conclusions for the three inconclusive results were satisfactory, requesting a new sample and recommending prophylaxis until further analysis.
Summary/Conclusions: The EQA2016 workshop demonstrates high reliability of routine fetal RhD genotyping and underlines its utility for targeted use of prophylaxis. Our future aim will be to set up a regular external quality assurance scheme open for participation by any laboratory engaged in noninvasive fetal RhD genotyping.

P-579

USE OF NON INVASIVE PRENATAL FETAL BLOOD GROUP GENOTYPING IN THE MONITORING OF ALLO-IMMUNIZED PREGNANT WOMEN: EXPERIENCE OF THE FRENCH NATIONAL CENTER FOR PERINATAL HEMOBIOLOGY (CNRHP)

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Background: The French "Centre National de Référence en Hémobiologie Périnatale" (CNRHP) is dedicated to biological and clinical diagnosis and treatment of feto-

maternal red blood cells incompatibilities. Maternal-feto blood group incompatibility is common and may result in haemolytic disease of the fetus and newborn (HDFN). This disease is characterised by anemia and hyperbilirubinemia which may lead to fetal hydrops, kernicterus or death. Three antibodies are associated with severe fetal disease: anti-RH1, anti-RH4 and anti-KEL1. High concentration of anti-RH3 can too led to HDFN during the third pregnancy trimester. Since the discovery of free fetal DNA into peripheral maternal blood, non-invasive prenatal determination of fetal *RHD* genotype on maternal blood is used in the management of pregnancies of RH-1 women.

Aims: Review of non-invasive fetal genotypes used in our reference center in determining of the feto-maternal RH1, KEL1, RH4 or RH3 incompatibility status in order to spare a specific antenatal monitoring.

Methods: To identify fetuses at risk for HDFN, our laboratory uses 4 analysis from peripheral maternal blood: (i) non-invasive fetal *RHD* genotyping using Free DNA fetal kit RHD® CEIVD from *Jacques Boy,* (ii) non-invasive fetal *KEL1* genotyping using a homemade method, (iii) and (iv) non-invasive fetal *RHc* or *RHE* genotyping using an adapted published method (Finning *et al.* Transfusion, 2007, 47: 2126–33). Fetal genotype results were compared with the phenotype of the red blood cells of the babies at birth.

Results: Over six years in our reference center,

- 1322 non-invasive fetal *RHD* genotypes from allo-immunized anti-RH1 women were done (1048 positive fetuses, 43 undetermined, 30 negative non-confirmed and 201 negative confirmed). The test has got a sensibility of 98.4%, a specificity of 95.3% and a negative predictive value of 100%.
- 272 non-invasive fetal *KEL1* genotypes from allo-immunized anti-KEL1 women were done (87 positive confirmed fetuses, 23 undetermined, 27 positive non-confirmed, 16 negative non-confirmed and 119 negative confirmed). The test has got a sensibility of 100%, a specificity of 90.9% and a negative predictive value of 100%.

Over 11 months in our reference center,

- 41 non-invasive fetal *RHc* genotypes from allo-immunized anti-RH4 women were done (31 positive foetuses and 10 negative confirmed). The test has got a sensibility of 100%, a specificity of 100% and a negative predictive value of 100%.
- 12 non-invasive fetal *RHE* genotypes from allo-immunized anti-RH3 women were done (7 positive foetuses and 5 negative confirmed). The test has got a sensibility of 100%, a specificity of 100% and a negative predictive value of 100%.

For 20.6% of the allo-immunized women, the pregnancy was compatible and no specific antenatal monitoring was necessary.

Summary/Conclusions: Non-invasive fetal red blood cell genotype is a powerful tool to diagnose a feto-maternal red blood cells incompatibility and allows to legitimize a costly and heavy specific antenatal monitoring.

P-580

PRENATAL GENOTYPING AND ITS IMPLICATION IN THE PREDICTION OF FETAL D STATUS IN A MULTI-ETHNIC POPULATION

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Background: Non-invasive fetal *RHD* genotyping has become a significant aid to the management of pregnancies at risk for hemolytic disease of the fetus and newborn (HDFN) and also to avoid antenatal anti-D prophylaxis in pregnant women carrying an RhD-negative fetus. Most protocols involve amplification of two or three exons to avoid obtaining false positive results with the more common variants of *RHD.* It is important that false positives do not result from the presence of the inactive African genes *RHDΨ* and *RHD-CE-D*^{S}.

Aims: Considering the genetic background of the Brazilian population, we evaluated the accuracy of fetal *RHD* genotyping by the analysis of three regions of the *RHD* gene in maternal samples and in cell-free fetal DNA from maternal plasma.

Methods: Genomic DNA from 10 serologically D-negative pregnant women was *RHD* genotyped. Real time multiplex PCR was performed in triplicate targeting the *RHD* gene exons 4, 5 and 7 in cell-free DNA, with exons 4 and 5 discriminating against detection of *RHD* and *RHDΨ*.

Results: Of the ten maternal samples analyzed, 7 samples were negative for exons 4, 5 and 7, one sample was positive for exons 4 and 7 and two samples were positive for exons 4, 5 and 7. *RHD* genotyping performed by RHD BeadChip (Immucor, Warren, NJ), revealed that the seven samples negative for exons 4, 5 and 7 had the *RHD* deletion and the sample positive for exons 4 and 7, was homozygous for *RHDΨ*. One of the samples with positive results for exons 4, 5 and 7 had one allele containing *RHDΨ* and one allele with *DIIIa-CE(4–7)-D* inactive genes and one sample was *RHD*DAR*. Real-time PCR showed 7 *RHD*+, 1 *RHD*- and 2 inconclusive results.

Summary/Conclusions: Although the *RHD* deletion was the most common genetic mechanism responsible for the RhD-negative typing in the samples evaluated, we also found the *RHDΨ* and the *DIIIa-CE(4–7)-D* hybrid gene. The sample typed D-negative with the compound heterozygote *RHDΨ/DIIIa-CE(4–7)-D* genotype showed discrepant results for exon 4 (*RHDΨ*+) and exons 5 and 7 (*RHD*+) and could be classified as RhD+ if only exons 5 and 7 of *RHD* had been analyzed. The knowledge of the genetic background of the population can reduce the number of inconclusive results when investigating fetal *RHD* status. This knowledge helped us on the development of a feasible protocol to determine fetal *RHD* status in our admixed population targeting *RHD* exons 4, 5 and 7 simultaneously.

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RISK ASSESSMENT FOR RH-1 PREGNANT WOMEN WHEN A NEGATIVE NON-INVASIVE FOETAL RHD GENOTYPING RESULT IS NOT CONFIRMED

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Background: Maternal-feto blood group incompatibility is common and may result in hemolytic disease of the fetus and newborn (HDFN). This disease is characterized by anemia and hyperbilirubinemia which may lead to fetal hydrops, kernicterus or death. Three antibodies are associated with severe fetal disease: anti-RH1, anti-RH4 and anti-KEL1. Although the widespread use of RhD immune globulin has resulted in a major reduction in the incidence of RhD immunisation in pregnancy, the maternal anti-RH1 allo-immunisation is the most common cause of feto-maternal red blood cells incompatibility resulting in HDFN. Many laboratories worldwide provide non-invasive fetal *RHD* genotyping as a routine service to help the practitioners to greatly improve the accuracy follow-up in pregnant women anti-RH1 allo-immunized or to restrict the use of anti-D immunoglobulins only to women bearing an RHD+1 fetus.

Aims: Risk assessment for RH-1 pregnant women when a negative non-invasive foetal *RHD* genotyping result is not confirmed when the presence of free fetal DNA is not tested.

Methods: Non-invasive fetal *RHD* genotyping were done using Free DNA fetal kit RHD® CEIVD from *Jacques Boy* which allows amplification of *RHD* exon 10, 7 and 5 from peripheral maternal blood. Furthermore, for RH-1 pregnant women carrying non-functional *RHDpsi* allele, amplification of *RHD* exon 6 was used. Non-invasive fetal *RHD* genotyping results from 1,477 RH-1 pregnant women were compared with or without confirming negative fetal RHD results. Fetal genotype results were compared with the fetal *RHD* genotype determined on amniotic cell or the phenotype of the red blood cells of the babies at birth.

Results: If no confirmation of negative test is done, non-invasive fetal *RHD* genotyping has got a sensibility of 98.4%, a specificity of 95.4% and a negative and positive predictive value of 98.5% and 99.6% respectively (5 false negative). If a confirmation of negative tests is done, non-invasive fetal *RHD* genotyping has got a sensibility of 98.3%, a specificity of 95.3% and a negative and positive predictive value of 100% and 99.6% respectively (0 false negative).

Summary/Conclusions: When negative tests are not checked, the negative predictive value of non-invasive fetal *RHD* genotyping is only 98%. This rick is may be allowed for non allo-immunized RH1 women, but it is not acceptable for allo-immunized RH1 women.

P-582

NON-INVASIVE PRENATAL DIAGNOSTICS OF HPA-1A IN POLAND

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Background: HPA-1a antigen may cause maternal alloimmunization and is responsible for ~85% of fetal/neonatal alloimmune thrombocytopenia (FNAIT). Non-invasive prenatal diagnostics (NIPD) of *ITGB3* encoding the HPA-1a is the most effective technique for determining feto-maternal incompatibility. For mothers with anti-HPA-1a antibodies it is important to determine fetal genotype to help in the decision concerning antenatal treatment.
Aims: Summary of HPA-1a NIPD in HPA-1a negative women, performed in Poland as part of HPA-1a screening program "PREVFNAIT" (the Project Agreement No. PolNor/203111/69/2013).
Methods: DNA was isolated (easyMag, Biomerieux) from 328 plasma samples from 299 HPA-1a negative pregnant women, collected into EDTA vacutainer tubes and transported up to 4 days; then digested with *MspI* enzyme and examined by real-time PCR for *HPA-1a* in triplicates and *CCR5* as control gene on LCII 480 (Roche Diagnostics Ltd.) according to Scheffer et al.
Results: In 281/299 cases the HPA-1a status of a neonate was available. In one case, it was not possible to perform NIPD because of the presence of HPA-1a variant in maternal genome. In 228/229 cases of women carrying an HPA-1a-positive fetus real-time PCR gave HPA-1a positive results in all three replicates except one case (mean Ct 36.0 ± 1.7SD). In 44/51 cases of mothers carrying an HPA-1a-negative fetus there was no amplification in at least 2 (out of 3) PCRs (mean Ct 44.1 ± 1.9SD). In 22 cases collected twice at 16–20 and 28 week of gestation the test gave HPA-1a positive results in all three replicates with mean Ct value about 37.45 ± 1.5SD and 35.39 ± 1, respectively. In 21 cases of mothers with anti-HPA-1a antibodies, NIPD done in 28th week determined 2 fetuses as *HPA-1a* negative (10%).
Summary/Conclusions: Real-time PCR is a highly reliable method for predicting fetal *HPA-1a* status at 28 week of pregnancy. Since the decisions on treatment options of FNAIT should be undertaken early in pregnancy, NIPD test improvements for use at earlier weeks of gestation would be beneficial.

P-583

NON-INVASIVE FETAL PLATELET GENOTYPING TO MANAGE HIGH RISK PREGNANCY OF FNAIT: A LARGE COHORT STUDY

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Background: Fetal and neonatal alloimmune thrombocytopenias (FNAIT) are caused by maternal antibodies against specific fetal platelet antigens inherited from the father and different from maternal antigens. It occurs at an estimated frequency of 1 in 1,000 live births in Caucasians and is the commonest cause of severe isolated thrombocytopenia in the fetus and newborn. The most severe consequence of FNAIT is intracranial hemorrhage, leading to death or neurologic sequelae in approximately 10% of symptomatic FNAIT cases. In the absence of screening programs, the first child with FNAIT is generally identified postnatally. Currently, fetal platelet genotyping is performed using invasive procedures with a risk of bleeding and miscarriage.
Aims: In our laboratory, only 30% of FNAIT cases are related to feto-maternal HPA-1 incompatibility. Therefore, we developed a new method for non-invasive fetal platelet genotyping using droplet digital PCR (ddPCR), which is widely applied in clinical practice since this technique is very sensitive and also allows detection of several rare alleles. We focused on target amplification of specific regions carrying polymorphism of 4 platelet antigen systems HPA-1, -3, -5 and -15 which are implicated in more than 95% of FNAIT and for some of them, serological tests failed to detect alloantibodies.
Methods: A large cohort of pregnant women with a previous history of FNAIT was investigated. We collected plasma samples from 38 women (6–35 WG) carrying fetus at risk of FNAIT. Fetal platelet genotyping was performed on cell free DNA extract from 54 maternal plasma. To exclude false-negative results caused by the lack of fetal DNA in maternal plasma, the methylation status of *RASSFA1* gene promotor was used as an internal control.
Results: Results showed that 9/38 (24%) pregnant women were compatible with their fetus, 17/38 (45%) were incompatible in one HPA system including 7 cases incompatible in only HPA-1, 7/38 (18%) in two HPA systems and 5/38 (13%) in three combined HPA systems. These predicted fetal HPA genotypes by ddPCR were confirmed in 24/38 (63%) FNAIT cases either by HPA genotyping performed on amniocentesis or by blood platelet phenotyping after birth. In the other cases, we will collect samples once pregnant women have given birth. Fetal DNA fraction in maternal plasma was estimated in all samples by using a reliable marker; it ranged from 0.5% to 26% of maternal circulating DNA and increased with gestational age.
Summary/Conclusions: This study strongly suggests that non-invasive fetal HPA genotyping using ddPCR appears as a safe and reliable method devoid of risk for the fetus. This technique allows early diagnosis of feto-maternal platelet incompatibility and could be implemented in routine clinical testing because of its high sensibility and specificity. Thus, non-invasive platelet genotyping appears attractive for clinical and therapeutic patient management. Indeed, it may provide a pregnancy risk evaluation, facilitate availability of appropriate phenotyped platelet concentrates at birth, and help preventing complications and unnecessary interventions such as IVIg implementation or serological flow-up during pregnancy in the absence of incompatibility.

P-584

NON-INVASIVE FETAL PLATELET AND RED CELL BLOOD GROUP GENOTYPING WITH THE USE OF TARGETED MASSIVELY PARALLEL SEQUENCING OF MATERNAL PLASMA CELL-FREE DNA

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Background: In pregnant women with a history feto-maternal incompatibility (fetal and neonatal alloimmune thrombocytopenia (FNAIT) or hemolytic disease of the newborn (HDN)), fetal human platelet antigen (HPA) or blood group genotyping is required to determine whether the fetus is at risk and whether prenatal interventions are required.
Aims: Published methods for non-invasive genotyping of fetal blood groups using maternal plasma cell-free DNA do not provide internal controls for exclusion of false-negative results.
Methods: Cell-free DNA was isolated from plasma of 6 pregnant women with a history of FNAIT due to anti-HPA-1a (4 cases), anti-HPA-5b or anti-HPA-15a and 1 K- and Jka- negative and 3 RhD-negative pregnant women with history of HDN and/or irregular antibodies. The gestational age at the time of blood sampling was 21 weeks (median; range 15–32). A primer panel was designed to target sequences flanking single-nucleotide polymorphisms (SNPs)/exonic regions of ITGB3 (HPA-1), ITGA2B (HPA-3), ITGA2 (HPA-5), CD109 (HPA-15), RHD, RHCE, KEL (Kk), ACKR1 (FY), SLC14A1 (JK), SLC4A1(DI), GYPA (M/N), GYPB (S/s), and SRY. These regions and 14 anonymous SNPs were massively parallel sequenced by semiconductor technology.
Results: The implicated non-maternal fetal blood groups (K, Jka, RHD, HPA-1a, HPA-5b, HPA-15a) were correctly identified in all cases. The counting of non-maternal sequences of Y chromosomal regions and autosomal SNPs allowed quantification of fetal load and served as internal control for the presence of fetal DNA. The fractional fetal DNA concentration was 12.67% (mean; range 4.4% to 27.14%).
Summary/Conclusions: We propose this method as a universal tool for reliable non-invasive detection of fetal blood group polymorphisms that are frequently involved in FNAIT and HDN.

P-585

SEX-DETERMINATION BY QPCR OF Y-CHROMOSOME REPETITIVE SEQUENCE (YRS) IN CELL-FREE FETAL DNA (CFFDNA) FROM MATERNAL PLASMA

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Background: CffDNA provides non-invasive prenatal tests (NIPT) using a blood sample from the pregnant woman to determine if the fetus has a Y-chromosome.

Knowledge of the sex of the fetus is helpful in cases with a risk of a sex-linked genetic disease like Duchenne muscular dystrophy and Hemophilia. This study focuses on performing quality assurance of a non-invasive antenatal sex determination analysis using a highly repetitive sequence on the Y-chromosome, YRS. The YRS-analysis has a high accuracy and sensitivity compared to other tests.

Aims: This study focuses on performing quality assurance of a non-invasive antenatal sex determination analysis. The aim of the study is to validate the accuracy and sensitivity of the YRS analysis for early sex determination using qPCR and cffDNA.

Methods: First-trimester blood samples from 285 pregnant women were analyzed for the repetitive sequence YRS on the Y-chromosome at the Laboratory of Blood Type Genetics, Copenhagen University Hospital, using QIAsymphony for DNA purification. Cell-free DNA was purified from 1 ml of plasma and eluted in 60 µl. Triplicates of 10 µl were analyzed by qPCR. GAPDH was used as control for total DNA. Predicted sex was compared with the phenotypic sex of the baby.

Results: A universal standard curve was made ranging from 3.3 pg DNA per PCR reaction to 3,300 pg DNA per PCR reaction. The LOD for the analysis was 0.5 geq per PCR reaction. It was possible to detect YRS from week 5 (n = 11) and to quantify YRS from week 6 (n = 35). One sample out of 285 first-trimester blood samples gave a false result. The sample was collected at week 4, and the result of the test was negative, even though fetus was male.

Summary/Conclusions: The antenatal sex-determination by qPCR of YRS in cffDNA from maternal plasma was shown to be a very robust analysis and can be used for non-invasive prenatal sex determination from gestational age of 5 weeks. This study shows that qPCR can be used to detect very small amounts of cffDNA. The analysis is currently used for antenatal RHD determination. The analysis of cffDNA using qPCR could be used for many other targets relevant to fetal and maternal immunology, e.g. it could be developed to be used for HPA-1a analysis to avoid fetal and neonatal alloimmune thrombocytopenia (FNAIT).

P-586

RED BLOOD CELL ANTIBODIES AND HEMOLYTIC DISEASE OF THE FETUS AND NEWBORN IN ISRAEL – WHAT IS THE EVIDENCE?

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Background: Hemolytic disease of the fetus and the newborn (HDFN) is a severe form of anemia caused by maternal antibodies against fetal red blood cells, which if untreated, can cause intrauterine and perinatal morbidity and mortality. HDFN can be caused by antibodies against the RhD antigen, ABO and other red blood cell (RBC) antigens. Maternal alloantibodies are acquired mainly via fetomaternal hemorrhage or allogeneic blood transfusions.

The Israeli Ministry of Health (MOH) recommends performing an antibody screen at least once during each pregnancy and if positive, the antibody must be identified. These guidelines recommend antibody screening only for Rh negative women. Data concerning the prevalence of isoimmunization among pregnant women at the national level is lacking.

Aims: To collect data concerning the prevalence and characteristics of isoimmunization among Israeli pregnant women and their newborns during an entire year.

Methods: A retrospective survey of all women who gave birth in hospitals between January to December 2011 was performed by the Israeli HDFN Transfusion Medicine Study Group using an excel sheet template which was sent to all relevant blood banks (n = 27) in Israel. The study was approved by each participant hospital's IRB. The study population included all women who gave birth in one of the participating hospitals (n = 15) for whom there were ABO, RhD, antibody screen and antibody identification data. Antibody titer, phenotype of the father's RBC, direct antiglobulin test of the newborn, the need for intrauterine or exchange transfusions were also reported if available.

Results: During 2011, there were 166,296 births in Israel, 106,786 (64%) of them in the participating hospitals. A positive antibody screen was detected in 4,855 (4.6%) women. Antibodies were identified in 873 (0.8%) of the women, 634 (73%) were RhD positive and 239 (27%) RhD negative [131/239 (55%) had anti D ≥ 1:8]. Excluding anti-D, the common clinically significant antibodies were: anti-E 181 (21%), anti-K 135 (15%) and anti-c 91(10%). Multiple alloantibodies were observed in 114 (13%) women. Nine newborns of mothers with alloantibodies to c (3), C (1), D (1), E (1), C+D (1), c+E (twins) were transfused. One newborn whose mother had anti-s required an exchange transfusion.

Summary/Conclusions: Alloantibodies were observed in 0.8% of the pregnant women regardless of their RhD status, mainly to antigens of the Rh and Kell blood group systems. Clinically significant HDFN requiring transfusion occurred in 10/873 (1%) newborns. The presented data include 64% of births in Israel during 2011 and support screening all pregnant women for alloantibodies.

P-587

ANTI P AND ANTI PP1P^{K} ALLO-ANTIBODIES AS A CAUSE FOR RECURRENT PREGNANCY LOSS – SOMETIMES YOU WIN AND SOMETIMES YOU LOSE

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Background: P and P^{k} blood group antigens belong to the GLOB (028) and P1Pk (003) blood group systems, respectively. They are both glycolipid structures located on the red blood cell membrane of almost all individuals. Naturally occurring anti-P or anti PP1P^{k} antibodies, present in the serum of individuals with the rare phenotypes P^{k} and p, respectively, have been associated with acute intravascular hemolytic transfusion reactions and higher frequency of early spontaneous abortions but not with clinically significant hemolytic disease of the fetus and newborn.

Aims: To report on the pregnancy management and diverse outcomes in two young women with anti P and anti PP1P^{k} antibodies.

Methods: Anti-P, anti PP1P^{k} antibody titers and pregnancy outcomes were reviewed in 2 cases of women with these rare allo-antibodies.

Results: Case 1: A 20-year old primigravida presented with a pregnancy loss at 14 weeks of gestation. Upon workup, an A+, P_1^{k} blood type was detected, with an anti-P antibody titer of 1:256. During her second pregnancy, plasmapheresis treatments were given thrice weekly since week 5 of gestation. Despite low titers (below 1:16) of anti P antibodies, the pregnancy ended at week 17, again due to a spontaneous abortion. In her third pregnancy, intravenous immunoglobulin (IVIG) and plasmapheresis treatments were given alternately thrice weekly since week 5 of gestation. Antibody titers were kept below 1:16. At 23 + 5 weeks of gestation, after being involved in a car accident, the patient presented with placental abruption and disseminated intravascular coagulation, ultimately resulting in fetal death. The patient received 4 units of compatible thawed red packed cells from the rare blood inventory of the Israeli National Blood Services (MDA-NBS), 20 units of cryoprecipitate and 7 units of fresh frozen plasma. Her fourth pregnancy was triplet and it also ended with spontaneous abortion at week 12. The antibody titers failed to decrease below 1:128, in spite of IVIG and plasmapheresis treatments since week 5 of gestation. With no preventive treatments due to the lack of health insurance coverage, the fifth, sixth, seventh and eighth pregnancies all ended at 14–16 weeks of gestation. Case 2: A 26-year old primigravida was referred to the Rambam Maternal-Fetal Division at 22 + 5 weeks of gestation due to a previously established p blood type with an anti PP1P^{k} titer of 1:32. Her antibody titer did not increase until the day of delivery of a healthy baby girl at 38 weeks of gestation. The newborn had P_2 (P1 neg PP^{k} pos) phenotype with negative DAT and normal hematocrit values. Due to a low post-labor hematocrit level, the mother received one unit of thawed autologous packed red blood cells.

Summary/Conclusions: In the cases reported herein, anti P and not anti PP1PK antibodies were the solitary cause of early and recurrent pregnancy loss. The high risk of bleeding complications associated with both pregnancy and plasmapheresis emphasize the crucial role of preemptive cryopreservation of autologous blood units in women with these rare but clinically meaningful antibodies.

Clinical transfusion

Neonatal and pediatric transfusion

P-588

FETOMATERNAL RED BLOOD CELLS ALLOIMMUNIZATION AND UNIVERSAL CORD BLOOD SCREENING AT BIRTH: ONE-CENTER EXPERIENCE

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Background: Fetomaternal red blood cells (RBC) alloimmunization is a mother's immune response to allogenic foetal antigens and may result in a haemolytic disease of the new-born (HDN). New-born blood grouping combined with mother's grouping can detect fetomaternal incompatibilities. Additionally, cord blood screening at birth by direct antiglobulin test (DAT) allows rapid detection of eventual mother's alloimmunization and antibody transfer to the new-born.
Aims: The aim of this retrospective study was to analyze the incidence and causes of positive cord blood DAT at birth and its relation to HDN development. We aimed also at evaluation of the importance of blood grouping and universal cord blood DAT screening for HDN risk assessment and its impact on the clinical management of the HDN.
Methods: Laboratory data from Erasme Hospital, ULB, Brussels, of new-born cord blood and mother samples was collected from January 2005 to December 2015. We analyzed the following data: cord blood DAT results, RBC elution test result, infant/maternal ABO, Rh and Kell grouping, antibody screening and antibody identification in cases of maternal alloimmunization, and neonatal bilirubin level. A retrospective review of the charts of babies with a positive cord blood DAT was also performed.
Results: A total of 21 562 new-born cord blood samples were included. The sex ratio was of 1.06. A and O groups were the most common in the ABO system and CcDee phenotype was the most common Rh phenotype. ABO fetomaternal incompatibility was assessed in 18.9% of our cohort with a higher risk of HDN in blood group A or B babies born from group O mothers. The risk of RhD fetomaternal incompatibility was estimated at 65.6% (RhD positive babies born from RhD negative mothers). Positive DAT frequency was 7.2% (n = 1,549) in our cohort. Analyzing bilirubin level, a significant difference was found between babies with negative DAT and those with positive DAT, with mean and median values in the negative COD group higher than those in the positive COD group ($P < 0.05$). In the DAT positive group, several antibodies were identified by the RBC elution test: Anti-A, B, D, E, S, Kell, Jka, Jkb and Tja. Additionally, a significant difference was found between bilirubin levels in babies with anti-D alloimmunization and those with other types of alloantibodies. Phototherapy and exchange transfusion were performed in 30.4% and in 2.8% respectively, in positive DAT newborns, and no death related to the HDN was recorded during the study period.
Summary/Conclusions: Our results show the importance for alloimmunization risk assessment by universal grouping and maternal antibody screening. The DAT result was not correlated to the bilirubin level, HDN being one of reasons for neonatal hyperbilirubinemia. The association of lower bilirubin levels with the presence of anti-D in the eluate of DAT positive cord blood sample can be explained by passive transfer of anti-D immunoglobulins related to the HDN prevention and not to alloimmunization. Early detection of HDN by DAT and eluate allowed us a highly efficient HDN management by active surveillance, intensive phototherapy or transfusion with no infant deaths related to HDN.

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ANALYSIS OF PEDIATRIC ADVERSE REACTIONS TO TRANSFUSIONS (APART)

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Background: Children are physiologically and biochemically different from adults. A recent single center study reported a significantly higher incidence of transfusion reactions among pediatric patients compared to adults (6.2 vs 2.4 per 1,000 transfusions) (Oakley, Transfusion, 2015). There is no multi-institutional study that has examined the differences in the frequency, type, and severity of transfusion reactions in pediatric vs adult patients in the United States.
Aims: This study aims to expand the knowledge on reactions to blood products in pediatric patients. The goal is to use hemovigilance data from multiple pediatric institutions to further characterize differences between pediatric and adult patients regarding adverse transfusion responses.
Methods: This is a retrospective analysis of hemovigilance data from 9 children's hospitals and 35 adult hospitals from January 2009 through December 2015. Included were pediatric (under age 18) and adult (age 18 and over) patients who had a reported reaction to transfusion of any blood component. Reactions were categorized according to standardized definitions by the Centers for Disease Control National Healthcare Safety Network Hemovigilance Module. Information collected included the total number of transfusions by year at each hospital, transfusion reactions by year of transfusion, patient age, final diagnosis (reaction type), severity of reaction, imputability of reaction to transfusion, component type, and symptoms. The aggregate denominator was calculated from hospitals reporting either pediatric specific or adult specific data for total transfusions per year. Adult hospitals which reported only adult adverse reactions and provided adult denominator data were included in the study. Pediatric hospitals were classified as such provided their institution type was reported as a children's facility. Rates are reported as per 100,000 transfusions.
Results: There were a total of 3,822 reported transfusion reactions from 1,222,869 transfused components during the study period. This consisted of 1,402 pediatric patient reactions to 260,664 components transfused and 2,420 adult patient reactions to 962,205 components transfused. Pediatric patients had an overall higher rate of reactions compared to adults; 538 vs 252 per 100,000 transfusions. Subtype analysis showed a higher rate for pediatric compared to adult patients for red blood cell (RBC) (577 vs 278 per 100,000) and platelet (833 vs 358 per 100,000) transfusions ($P < 0.001$). Statistically higher rates of allergic reactions, febrile non-hemolytic reactions, and acute hemolytic reaction (AHTR) types associated with RBC transfusion were observed in pediatric patients. The higher reaction rate was most pronounced with AHTRs to red blood cells, which had a pediatric rate of approximately 10 times that of the adult group (9.03 vs 0.90 per 100,000, $P < 0.001$). Adults had a higher rate of all types of delayed reactions and transfusion associated circulatory overload.
Summary/Conclusions: Pediatric patients had two times the rate of transfusion reactions compared to adults. The nationally reported data on reaction rates for the United States are consistent with our findings in adults but much lower than our observed rates for pediatric patients (Harvey, Transfusion, 2015). Future studies are needed to address the differences in reaction rates, and to further address blood stewardship practices in the pediatric patient population.

P-590

FERRIC CARBOXYMALTOSE IN CHILDREN LESS THAN 14 YEARS OF AGE

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Background: Intravenous iron may be used when there are contraindications to oral iron therapy, issues with compliance, tolerability or lack of efficacy with oral iron preparations, when rapid iron repletion is required, or as adjunctive therapy with erythropoietin-stimulating agents. Ferric carboxymaltose is being used with increasing frequency due to its rapid administration, efficacy and safety profile. In Australia is only licensed by the Therapeutic Goods Administration for use in children aged 14 years and older. Evidence for its use in children less than 14 years is limited.
Aims: To review the safety and efficacy of ferric carboxymaltose in children aged less than 14 years at the Royal Children's Hospital, Melbourne, Australia.
Methods: Retrospective study of all ferric carboxymaltose infusions performed between September 2013 and May 2015 in children. Data on the following variables were collected: patient demographics, indication, dose, adverse effects, and efficacy as assessed by Hb, MCV and ferritin.
Results: Sixty-five episodes of ferric carboxymaltose administration to 60 children were analysed; five children received two infusions. Median age at administration 9.3 years [IQR 3.4–12.4]. Median dose was 19 mg/kg [IQR 13–20]. Gastroenterological disease was the most common indication 30/65 (46%), followed by renal disease 11/65 (17%), dietary iron deficiency anaemia (IDA) unresponsive to oral iron 8/65 (12%) and IDA secondary to menorrhagia 5/65 (8%). No life-threatening complications were reported. One episode of extravasation was documented and two children required multiple attempts to secure IV access. Three infusions were associated with mild

adverse effects, which may have related to the iron infusion or underlying clinical disease. IV ferric carboxymaltose resulted in a significantly improved Hb and MCV ($P < 0.001$) whilst increase in ferritin did not meet statistical significance ($P = 0.06$).
Summary/Conclusions: Our study supports the safety and efficacy of ferric carboxymaltose as an IV iron therapy for use in children under 14 years. Important benefits of ferric carboxymaltose include: rapidity of infusion, shorter hospital stay and less patient inconvenience.

P-591
THE EFFICIENCY OF ANTIGEN-POSITIVE PLATELET TRANSFUSION IN NEONATAL THROMBOCYTOPENIA CAUSED BY ALLOIMMUNIZATION TO THE HPA-1A ANTIGEN

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Background: Fetal and neonatal alloimmune thrombocytopenia (FNATP) is the result of maternal alloimmunization against paternally inherited specific platelet antigens (HPA) of the fetus, during pregnancy. FNATP is a rare but potentially life-threatening disorder. The incidence is estimated to 1:1,000 to 1:2,000 births in white populations. Clinical course varies from asymptomatic to severe cases with intracerebral hemorrhage (ICH) resulting in death or long-term disability. In most cases ICH develops due to HPA-1a alloantibodies. Serologic testing for FNATP in case of isolated thrombocytopenia in the newborn contributed considerably to timely detection of this disease. Platelet transfusions are needed in severe cases to prevent ICH. Current guidelines recommend transfusion of HPA compatible apheresis platelets or mother's washed and irradiated platelets. Because of approximately 10% of untreated neonates with FNATP are affected by cerebral hemorrhage in the first days of life, a liberal approach toward platelet transfusion is considered appropriate (threshold above 30×10^9/l).
Aims: The aim of this study was to retrospectively analyze laboratory and clinical data of eight newborns with FNATP due to anti-HPA-1a who received antigen positive platelets from random donors.
Methods: In the period from 2004 to February 2017, eight newborns with serologically proven anti-HPA-1a FNATP in the Croatian Institute of Transfusion Medicine, Department of platelet and leukocyte diagnostics and hemostasis were identified receiving 9 antigen positive platelet transfusions (subsequently genotyped as HPA-1aa).
Results: Five of 8 newborns were male and three female. Six of 8 were born to primiparous women. All neonates had skin hemorrhage (localized or generalized petechia) and two had intracerebral hemorrhage. Maternal antibody status was known in one of 8 newborns at the time of transfusion. Average platelet count before transfusion was 28×10^9/l (range 7×10^9/l to 34×10^9/l). Seven neonates received apheresis platelets in dose of 10 ml per kg BW and two received one single unit of platelet reach plasma (PRP) platelets. Five of 8 newborns showed an increase of platelets above 40×10^9/l (range 47×10^9/l to 87×10^9/l) after random donor platelet transfusions. Two newborns needed more than one transfusion. In addition to platelet transfusion, two newborns received immunoglobulins (IVIG), one received IVIG and corticosteroids and one corticosteroids only. All neonates reached full recovery.
Summary/Conclusions: Serologic testing for FNATP in case of isolated severe thrombocytopenia in the newborn contributed considerably to timely detection and favorable outcome. Random donor platelet transfusion is an acceptable approach in urgent situations, when antigen negative platelets are not readily available. In addition, intravenous immunoglobulins (IVIG) and corticosteroids can be given to prolong the survival of the incompatible platelets.

P-592
MASSIVE TRANSFUSION AT A TERTIARY PAEDIATRIC CENTRE

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Background: Neonatal and paediatric patients are a unique population when considering massive transfusion (MT) and critical bleeding. A number of age-related physiological variations exist, that may exacerbate the effect of critical bleeding and large-volume transfusions. In 2011 the National Blood Authority, Australia released guidelines on managing critical bleeding and massive transfusion and recommended hospitals develop a massive transfusion protocol. The Royal Children's Hospital (RCH), Melbourne, Australia is the state trauma centre and treats a diverse cohort of neonates and children with complex surgical and medical conditions. To support these vulnerable patients during instances of critical bleeding, a MT procedure (MTP) was developed with specific guidance around responsibilities, communication, laboratory sampling and weight-adjusted critical bleeding packs.
Aims: To retrospectively review all episodes of MT since implementation of the MTP with respect to key components in the procedure.
Methods: A retrospective review of all episodes of MT at RCH from September 2014 to June 2016. Data collected included: demographics, indication for MTP activation, volumes of blood components transfused, communication, documentation, and laboratory investigations.
Results: Twenty-eight MTP activations occurred between September 2014 to June 2016. Median age was 4 years (range 1 day–16 years) with 5/28 (18%) being <1 month of age. Median patient weight was 27.2 kg (range 2.1–80 kg). The most common indication for MTP activation was trauma 12/28 (43%) followed by emergency surgery 7/28 (25%). In 4/38 (14%) of activations there were problems with the laboratory samples including: coagulation samples not being collected, being clotted or over-filled or crossmatch specimen declarations not being signed. Tranexamic acid was received by 7/28 (25%). A median of 36 ml/kg RBC were transfused and the median RBC:FFP ratio was 1.4:1.
Most patients 22/28 (79%) were successfully resuscitated. Six children died, four were admitted with trauma, one underwent emergency surgery and one had a gastrointestinal bleed.
Summary/Conclusions: Critical bleeding and MT in neonates and paediatric patients occurred most commonly in the setting of trauma or emergency surgery. These children had considerable transfusion requirements and most were successfully resuscitated. Rarely patients with critical bleeding and MT activation did not receive any blood components; in the instance when there was rapid control of bleeding. Blood products received prior to the MTP activation were not captured by our study and this is an area of future review in order to accurately calculate transfusion ratios.

P-593
INTRAUTERINE TRANSFUSION IN OMAN: THE FIRST STEPS

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Background: Hemolytic Disease of the Fetus and the Newborn (HDFN) is a known cause of potentially fatal fetal hydrops. Intra Uterine Transfusion (IUT) is considered the only rescuing measure for the fetus. This procedure is done either intra-vascularly or intra-peritoneally, utilizing specifically prepared blood components. Not only it needs to be limited to tertiary care setting, but it also necessitates sophisticated skills, special measures for blood component preparation and a multidisciplinary team effort for its success.
Prior to 2012, cases necessitating IUT in Oman had to be sent abroad due to the lack of the needed skills and supportive services for such procedure in the country. In March 2012, and with collaborative efforts, the first IUT was performed in a patient with anti-D HDFN. Since then, a multidisciplinary program had been developed to support this procedure in all referred-in cases with fetal anemia.
Aims: Herein, we describe the first four-year' experience of running the first IUT program in Oman, the indications, details of the procedures, perinatal survival and neonatal outcomes.
Methods: A retrospective cross-sectional observational cohort study was conducted of all women undergoing IUT for fetal anemia between March 2012 and March 2016. Clinical, blood bank and neonatal data was reviewed. Results of antenatal blood bank antibody screening tests, obstetric history and indications for the IUT were assessed. Gestational age at the time of the first IUT performed on the mother, fetal status, number of procedures required, and procedure-related complications were examined. Finally, fetal and neonatal outcomes post-delivery were examined including fetal survival, gestational age at time of delivery, neonatal survival, short-term morbidity and neurodevelopmental outcomes.
Results: Total of 28 IUT procedures were performed in 13 fetuses of 11 women, 8 of which were multi-parous with past history of immune fetal anemia. Gestational

Transfused blood products during MT activation*							
	RBC	FFP	Platelet	Cryoprecipitate		RBC:FFP ratio	RBC:platelet ratio
Median ml/kg	36	19	9.5	6.5	Median ratio	1.4:1	3.3:1
Minimum ml/kg	0	0	0	0	Minimum ratio	0	0
Maximum ml/kg	139	162	60	15	Maximum ratio	7.5:1	10.5:1

*Does not include blood products administered prior to MT activation.

age at time of presentation ranged between 18 and 28 weeks. All procedures were supported with irradiated leuko-reduced group O negative red blood cells negative for the relevant antigens in which the patients had developed antibodies against. Indications for the IUT included immune mediated HDFN caused by anti-D (n = 6), combination of anti-D and anti-C (n = 4), anti-K (n = 1), anti-Jsb (n = 1), and non-immune hydrops (n = 1). Median hemoglobin levels at the start and the end of the procedures were 4.6 g/dl (range 1.5–10) and 12.8 g/dl (range 6–19) respectively. Number of procedures performed on the patients ranged between 1 and 4 per pregnancy. Total of 3/28 procedures were done via the trans-placental intra-cardiac route, 1/28 were trans-amniotic intravascular and 24/28 were performed through trans-placental intravascular route through the umbilical cord. Six procedures needed to be started with an exchange transfusion due to the severity of the fetal anemia. Eight fetuses survived with one had some neurological sequelae. One patient had 3 pregnancies been supported by IUT, with successful outcome in two of them.
Summary/Conclusions: In conclusion, IUT is a new successful addition to the obstetric management in Oman that necessitates multidisciplinary team effort, sophisticated skills and specific blood components. Success of such procedure requires collaboration and provision of needed supportive services.

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Abstract has been withdrawn.

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RETROSPECTIVE ANALYSIS OF NEONATAL BLOOD TRANSFUSION OVER PAST 6 YEARS IN A CHINESE HOSPITAL

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Background: Timely, appropriate and safe blood transfusion during and after labour and delivery can make the difference between life and death for many women and their newborns. This study was designed in order to understand the validity of transfusion to neonates, helping make more effort to carry out the routinely restrictive transfusion strategy in neonatal ward.
Aims: Through retrospective analysis of all blood transfusion cases in the neonatal ward of a Women's and Children's Hospital over past 6 years, to evaluate the neonatal blood transfusion data and provide a reference data for newborns in transfusion medicine.
Methods: Collating the blood transfusion data from the Blood Bank laboratory of West China Second University Hospital from January 2011 to December 2016, and the neonatal clinical information from neonatal ward at the same time. Parameters reported in this study include: transfused blood type, transfusion volume, transfusion aim, transfusion efficiency and disease diagnosis, etc. A total of 2,993 neonatal patients who were transfused blood over past 6 years were enrolled in this study.
Results: For these 2,993 newborns, there were 6,314 person-time transfused during the past 6 years, of which red blood cells were the main component transfused, accounting for 68.3%; Followed by plasma (23.1%), platelet (5.4%), and cryoprecipitate (3.2%). Neonatal hemolytic disease caused by blood type incompatibility between mother and infant, and unexplained high neonatal bilirubin concentration were the most direct reasons for neonatal blood change. Other important reasons for newborns requiring of component blood transfusion were premature infants, neonatal infections, pneumonia, sepsis, newborn thrombocytopenia, et al.
Summary/Conclusions: As the release of domestic two-child policy in China for the recent 3 years, more and more complicated severe neonatal diseases were found. Increasing neonates suffering dubious and acute diseases need blood transfusion for treatment. The medical science for newborns is developing rapidly, however, the blood transfusion amount and person-time in the neonatal ward is not less. Although the Blood Center is providing better services to ensure supplies of blood components, the shortages of blood resources still often occur. Scientific and reasonable, safe and effective transfusion strategies used in clinical neonatal practice, we still need to do more efforts.

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THE PREVALENCE OF RBC T ACTIVATION AND TRANSFUSION THERAPY EXPERIENCE IN SOUTHERN MEDICAL CENTER OF TAIWAN

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Background: Cryptantigen exposure by bacteria infection has been discussed well including T-antigen activation. T-activated RBCs (T-antigen) react with the *Glycine soja* and *Arachis hypogea* lectins; this type of activation is the most common to form polyagglutination. Because anti-T IgM antibodies exist in most adults' serum, quite a few reports about transfusion-associated hemolysis in infants with T-activated RBCs have been mentioned. So the lack of plasma or low-titre blood components are suggested for transfusion but the definition of low-titre has been various.
Aims: The aims of this study were to analyze the prevalence of RBC T activation in our institution and describe transfusion practice for infants showed RBC T activation.
Methods: the present study was also a retrospective study. Between January 2014 to February 2017, a total of 43 samples from 42 infants were tested for T-antigen using *Arachis hypogea*. If the test results were positive, medical and transfusion history were investigated. The blood products for infants who needed transfusion were Wash RBCs, low-titre anti-T/plasma-reduced with low-titre anti-T Platelet concentrates. The low-titre in present study defined as agglutination titers less than 8.
Results: Over 26 months, 5 of 43 samples had T -activated RBCs, the prevalence was 11.63%. Those 5 samples were from 4 infants who all were pneumonia cases, two of them (baby Y and baby D) received transfusion treatment. The diagnosis of baby Y was necrotizing pneumonia with multiple abscess. On 9th November 2014, she randomly transfused FFP 2U and Platelet concentrates 4U, Wash RBCs 1U initially, but the Platelet count dropped from 189 to 71 $10^3/mm^3$; therefore, totally 30U low-titre anti-T Platelet concentrates, 1U Wash RBCs and random FFP were given in the following 3 days. Her Platelet count increased to normal range (249 $10^3/mm^3$) then; however, due to her pathological conditions, her Hb rose temporarily after transfusing 2U Wash RBCs (from 7.6 to 11.6 g/dl) but decreased to around 9 g/dl till her discharge on 27th November. Baby D was diagnosed as pneumococcal pneumonia with pleural effusion, right necrotizing pneumonia with empyema. On 2nd January 2017, he received 2U random Platelet concentrates but the Platelet count dropped from 63 to 52 $10^3/mm^3$. Hence, he was given 20U plasma-reduced with low-titre anti-T Platelet concentrates, 2U Wash RBCs in the next 2 days, his Hb and Platelet count turned to normal range (9.2–13.7 g/dl; 63 to 152 $\times$ $10^3/mm^3$).
Summary/Conclusions: A large number of studies have described hemolysis with blood transfusion in T-activation infants, but also many reports suggest that it shouldn't be strict avoidance for giving plasma-containing blood components to T-activated patients. For clinical transfusion practice, providing specially prepared blood products for infants would be necessary according to our experience. For those infants who require FFP transfusion to correct a coagulopathy, low-titre anti-T FFP will be given in the future.

Therapeutic apheresis

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EVALUATION OF THE APOPTOTIC EFFECTS OF EXTRACORPOREAL PHOTOPHERESIS FOR THE TREATMENT OF CHRONIC GRAFT-VS-HOST DISEASE

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Background: Extracorporeal photochemotherapy (ECP) represents one of the most frequent therapeutic approach for chronic graft-vs-host disease (cGVHD). ECP consists of infusion of UVA-irradiated autologous peripheral blood mononuclear cells (PBMNCs) collected by apheresis and incubated with 8-methoxypsoralen (8-MOP). The mechanism of ECP has not been fully elucidated yet. Several authors have reported that the therapeutic effect of ECP is mainly achieved by the induction of cell apoptosis, influencing the function of dendritic cells and the induction of immune tolerance.
Aims: The aim of this study is to determine the cellular and molecular mechanisms undergoing the effectiveness of ECP treatment in patients with cGVHD in order to identify early potential markers of response to treatment.
Methods: Autologous PBMNCs were collected by a continuous-flow blood cell separator (COBE Spectra) and ECP was performed using the UVA Pit kit (Med Tech Solutions). PBMNCs from patients with refractory cGVHD were collected at different times: before (post-collection and after the addition of 8-MOP) and after (prior to reinfusion of the final product to the patient) UVA exposure. Patients' MNCs were cultured for 48 h to assess the kinetics of apoptosis at different time: externalization of phosphatidylserine was evaluated with a FITC-labeled Annexin V/propidium iodide (PI) apoptosis detection kit (Miltenyi). Furthermore, we determined intracellular proteins involved in the commitment, onset and induction of apoptosis with the Bio-Plex Pro RBM Apoptosis Assays (Bio-Rad).
Results: We evaluated 9 patients with refractory cGVHD, 7 males and 2 females (median age: 54 years; range: 34–65). All patients underwent ECP in combination with immunosuppressive therapy. Samples treated with 8-MOP and UVA irradiation showed a significant change ($P = 0.03$) in the percentage of cells positive for Annexin V 24 h after the procedure when compared to untreated samples. These changes increase 48 h after the treatment. We also observed a significant reduction of anti-apoptotic markers Bcl-xL/Bak ($P = 0.002$), Bax/Bcl-2 ($P = 0.013$), Mcl-1/Bak ($P = 0.020$) and Survivin ($P = 0.047$) in treated samples compared to untreated samples, 24 h after the ECP.
Summary/Conclusions: Our results showed that ECP induces apoptosis of MNCs with the main involvement of the Bcl-2 protein family. The reduction of the anti-apoptotic markers causes the release of cytochrome C into the cytosol, where it promotes apoptosome formation and final activation of caspase 3. Caspases exist as inactive zymogens in cells and undergo a cascade of catalytic activation at the onset of apoptosis. These mechanisms may explain the immunomodulatory properties of apoptotic cells induced by ECP treatment. An extensive understanding of these mechanisms may provide markers to evaluate response to treatment.

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INCREASING USE OF THERAPEUTIC APHERESIS AS A LIVER-SAVING MODALITY

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Background: Therapeutic plasma exchange (TPE) can be used for temporary support of liver function in patients undergoing early graft dysfunction after liver transplantation (LT). Even though TPE is effective for patients with early graft dysfunction, there are no consensus regarding the process of performing TPE.
Aims: In this retrospective clinical study, we analyzed the efficacy of therapeutic apheresis for patients with liver disease.
Methods: Between January 2011 and August 2016, 93 TPE procedures were performed for 26 patients in the hepatology department at a single hospital in the Republic of Korea. Clinical information including age, sex, diagnosis, and laboratory data were assessed through a retrospective chart review.
The anti-ABO isoagglutination IgM titer was checked by using a type A and type B 3% red blood cell (RBC) suspension (Ortho Clinical Diagnostics, Pencoed, UK) in saline with two-fold serial dilutions of patient serum at room temperature, followed by centrifugation and scoring for agglutination. The anti-ABO isoagglutination IgG titer was checked using a type A and type B 0.8% RBC suspension (Ortho Clinical Diagnostics) using a low-ionic strength/Coombs card (Bio-Rad, Murten, Switzerland) with two-fold serial dilutions of patient serum. The anti-ABO IgM/IgG titer was monitored every day starting from TPE until 2 weeks after LT. To reduce anti-ABO isoagglutination, a single dose of rituximab (300 mg/m^2/body surface area) was administered to all patients 2–3 weeks before LT. After 1 week of injections of rituximab, isoagglutination titration was monitored every day. If the IgG titer was higher than 1:256, then TPE was started on day 7 after injection of rituximab and TPE was performed every other day. If the IgG titer was lower than 1:256, then TPE was started on day 14 after rituximab injection.
When hyperbilirubinemia (>10 mg/dl) occurs in patients with early graft dysfunction, the physician requests TPE. TPE was performed every other day until the bilirubin level was <10 mg/dl. For patients with other diseases, we performed TPE when requested by the physician for patients with hyperbilirubinemia (>10 mg/dl), and the schedule was the same as for early graft dysfunction.
Results: From February 2015 to August 2016, 10 recipients planned to undergo ABOi LT. The median initial IgM and IgG anti-ABO titers were 1:16 (range, 1: 8–1:128) and 1:48 (range, 1:8–1:2,048), respectively. We performed preoperative TPE for 10 recipients (median number of sessions, 1.5; range, 1–11). Early graft dysfunction occurred after LT in 2.4% (8/320) of all LT patients at our hospital. However, more than half of these patients (n = 5; 62.5%) survived with the use of TPE. We performed TPE for 11 patients with either early graft dysfunction after LT or major hepatectomy. A median of three (range, 2–8) TPE sessions was performed. Among patients with early graft dysfunction, patients who underwent living donor LT had better survival rates (4/4; 100%) than those who underwent nonliving donor LT (0/3; 0%).
Summary/Conclusions: In our experience, therapeutic apheresis is associated with a good survival rate and is essential for liver support for patients with early graft dysfunction after LT or posthepatectomy liver failure and during preparation for ABOi LT.

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ROLE OF THERAPEUTIC PLASMA EXCHANGE IN ANTIBODY MEDIATED REJECTION IN LIVE RELATED RENAL TRANSPLANTATION

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Background: Renal transplantation is one of the treatment modalities available for end stage renal disease patients. Renal allograft rejection and its management have always been of concern.
Aims: To determine the effectiveness and predictors of response to therapeutic plasma exchange (TPE) in patients with antibody mediated rejection (AMR) of renal allograft.
Methods: A prospective study was conducted in the Department of Transfusion Medicine, Indraprastha Apollo Hospitals, New Delhi, India from 1st July 2014 to 31st December 2016 in live related renal allograft recipient (>18 years) with biopsy proven AMR (ABO group compatible). The study was approved by the institutional ethical committee. Clinical history, donor and graft details, management of AMR (TPE; rituximab; intravenous immunoglobulin-IVIg) along with laboratory parameters, patient and graft survival were noted. Patients undergoing first time renal transplants only were included in the study. As per our hospital policy, AMR treatment protocol included 1.5 plasma volume TPE (Haemonetics MCS plus; at least 5 consecutive sessions using reconstituted 5% human albumin and/or fresh frozen plasma) followed by

IVIg administration (dose 400 mg/kg × 3–5 doses). Rituximab (dose 375 mg/m²) was administered to patients not responding to TPE plus IVIg therapy.
Patients showing recovery of renal function with dialysis independence were considered as favorable response. Unfavorable outcome included patients showing persistent derangement in the renal function followed by dependence on dialysis post-transplant and/or graft nephrectomy or mortality.
Wilcoxon-rank sum test and Chi-square test were used for statistical analysis. P value less than 0.05 was considered significant.
Results: Of the 1,608 patients, 49 (37 males, 76%; 12 females, 24%) had biopsy proven AMR (3.04%). Mean age at time of rejection was 39.5 ± 13.3 years. A total of 281 TPE procedures were performed with an average of 5.73 TPE per patient. Two hundred three TPE were done among 38 patients receiving IVIg (Group A), while 78 for 11 patients receiving IVIg and rituximab (Group B).
Out of 49 patients, 38 (78%) showed favourable response (group A – 31; group B – 7). At 12 months of post-AMR, patient and graft survival was 84.2% and 81.5%, respectively. One patient was lost to follow up. Eleven (22%) patients had unfavourable response (group A – 7; group B – 4) with mortality among 3 patients.
Blood urea (P = 0.012) and serum creatinine (P = 0.038) levels at the time of rejection were significant predictors of response to TPE. Owing to higher baseline blood urea and serum creatinine levels among the said 11 patients with unfavorable response, more sessions of TPE were required leading to increased length of stay (P = 0.011) and yet no recovery of graft function was noted. On comparing Group A and B, rituximab had no effect on the outcome (P = 0.237).
Summary/Conclusions: Blood urea and serum creatinine values at the onset of rejection were important predictors for determining the response to TPE. TPE is an effective treatment modality and its combination with IVIg and/or rituximab is safe and effective for AMR.

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PARANEOPLASTIC NEUROLOGICAL SYNDROME (PNS): THREE CASE REPORTS WITH THERAPEUTIC PLASMA EXCHANGE (TPE)

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Background: Paraneoplastic syndromes affecting the nervous system are rare neurologic disorders caused by cancer but not ascribable to metastases or direct invasion. Onconeural antibodies and cytotoxic T lymphocytes are responsible for the immune response and usually occur as the first sign of the tumor or lead to its detection. 18.3% of patients harbored no antibodies. Classical PNS manifestations are subacute cerebellar degeneration, limbic encephalitis, paraneoplastic encephalomyelitis, opsoclonus-myoclonus syndrome and Lambert-Eaton syndrome. The tumors most commonly associated with PNS are those that express neuroendocrine proteins. Treatment includes antitumor and immunosuppressive therapy, including TPE.
Aims: Analyze the role of TPE in the treatment of Paraneoplastic Neurological Syndrome.
Methods: Retrospective and descriptive analysis of three case reports, evaluating: clinical manifestations, tumor association, onconeural antibodies, immunosuppression treatment, TPE protocol and patients outcomes.
Results: Case 1: 69 year old man with limbic encephalitis, ataxia, opsoclonus or myoclonus, bilateral retinopathy, anti -CV2 (+++) with a small cell lung cancer (SCLC) diagnosed after the PNS. First treated with corticosteroids × 5 days and immunoglobulin 0.4 g/kg/day IV × 5 days (IVIG); maintenance treatment with monthly IVIG during 3 years. After relapse and IVIG failure, a TPE program is initiated: 6 procedures, every other day, 1–1.5 TPV over a 2 weeks period, with complete neurological remission. Long term monthly TPE is required for maintenance of neurologic symptoms remission (2.5 years until nowadays). Continues with antitumor treatment without disease progression. Case 2: 69 year old man with Lambert-Eaton Syndrome, presented proximal muscle weakness of the lower extremities, hyporeflexia and autonomic dysfunction (impotence, constipation, hypohidrosis). Anti-Ri (+), Anti-PNMA2 (+), Anti-Amphiphysin (+), Anti-CV2 (+/-). Excluded SCLC and two years later diagnosed with a Non- Hogdkin B Lymphoma. First treated with pyridostigmine and then with monthly IVIG. Three months later, after limited response to IVIG, a TPE program is initiated: 8 procedures, carried out at 7–15 day interval, 1–1.5 TPV over a 2 months period, with partial neurological response. TPE was discontinued after starting treatment with 3,4-Diaminopyridine and 10 mg of prednisolone id, with no neurological relapses and with lymphoma control. Case 3: 73 year old female with limbic encephalitis 3 years after the diagnosis of lung adenocarcinoma and with bone metastases. Cerebral metastases and toxic or metabolic causes for limbic encephalitis were excluded. ON-Abs negative. First Immunosuppression treatment with methylprednisolone 1 g IV × 5 days and immunoglobulin 0.4 g/kg/day IV × 5 days with no response. TPE program is initiated: 6 procedures, every other day, 1–1.5 TPV over a 2 weeks period, with poor response. The patient died 1 month later, after an aspiration pneumonia.
Summary/Conclusions: Although optimum role of apheresis therapy is not established in the treatment of PNS (ASFA category III), in two of our patients there was a neurologic symptoms remission (total/partial). When there isn't a cancer remission a TPE maintenance protocol may be useful to avoid neurologic relapse. Since TPE was performed after the initial immunosuppression treatment has failed, with favorable response, we hypothesis if TPE used in the initial treatment protocol can be more effective in PNS control.

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ROLE OF THERAPEUTIC PLASMA EXCHANGE (TPE) IN PATIENTS WITH DRUG INDUCED LIVER INJURY

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Background: In the settings of acute liver failure, Therapeutic plasma exchange (TPE) provides a potential therapeutic option to temporarily support liver function and provide scope for regeneration. Drug-induced liver injury (DILI) remains a challenge for clinicians because of the lack of definitive diagnostic tests and treatment. Management of DILI includes prompt withdrawal of offending drugs and comprehensive supportive care.
Aims: To analyse the effect of therapeutic Plasma exchange on coagulopathy and liver function tests in patients with DILI and its role as supportive therapy
Methods: A prospective study conducted at Global Health City, Chennai from December 2015 to January 2017. Patients admitted with jaundice (S. Bilirubin >15 mg/dl) and diagnosed as Drug induced liver injury with history of recent drug intake were included in study after taking informed consent. One plasma volume TPE was performed using centrifugal cell separator and Central venous catheter as venous access. Vital parameters were monitored during TPE for any adverse effects. The pre and post-TPE laboratory data of the patients which included complete blood count, Coagulation parameters, Liver function tests, Renal function tests and serum electrolytes. The procedural details including estimated total plasma volume exchanged, number of repeated cycles or any complications associated with TPE were documented. The data was analysed statistically using SPSS software.
Results: Study included 10 patients (M:F 1:1) with average age 40 (17–67 years) admitted with recent onset of Jaundice, diagnosed as drug induced liver injury and possibilities of other aetiologies were ruled out. The primary diagnosis and drug history included pulmonary tuberculosis on ATT (n = 1), malarial fever on antimalarial drugs with Paracetamol overdose (n = 1), native medication for skin and rheumatic disorders (n = 5), Stanazol anabolic steroids (n = 1), carbamazepine for migraine (n = 1), Antibiotics Augmentin (n = 1). A total of 26 TPE procedures were performed with mean of 2.6 (1–5) TPE per patient. Average 4,956 ml (range 3,509–6,562) WB was processed using ACD anticoagulant. The mean Total Bilirubin on admission was 27.3 mg/dl (20.7–43.0) which significantly reduced to mean 16.5 (13.4–32.4) mg/dl post TPE. There was significant improvement in Direct Bilirubin (P < 0.001), INR (P < 0.05), Serum aminotransferases (P < 0.05) also while no significant change was seen in WBC count, platelet count and serum Creatinine. No adverse events were noted during or after TPE procedures. Hospital stay was mean 6.9 days (2–12 days). Patients had mean Total Bilirubin 11.1 mg/dl (8.3–15.2 g/dl) at the time of discharge. 9/10 patients recovered fully except one patient who was discharged against medical advice.
Summary/Conclusions: In view of our initial experience, TPE should be considered as an effective and safe liver supportive therapy for rapid recovery of patients with hyperbilirubinemia due to drug induced liver injury. Further randomised multicentric trials are required to assess the efficacy of TPE in DILI.

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RATIONALIZATION AND OPTIMIZATION OF THE THERAPEUTIC PLASMA EXCHANGE APPLICATION

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Background: Therapeutic plasma exchange (TPE) is a nonselective, nonspecific method used for the separation of circulating agents, an automated apheresis procedure with extracorporeal circulation aimed at reducing the concentration or elimination of pathogen from the blood plasma, to reimburse the removed volume with suitable liquid. TPE is used in a number indications, which are currently classified in to four separate categories. The category I includes diseases in which the TPE is standard – the primary treatment option. For disorders of category II, TPE is an additional, supportive therapy, usually in combination with other treatment modalities. In the category III are pathological conditions, in which the exact role of apheresis is not still safely defined – validity of TPE application, as well as the achieved effects are individual. The category IV includes diseases in which performing of TPE has not been proven effective.
Aims: Continuous introduction of new and more effective immunomodulatory and other medications, the indications for performing TPE and achieved effects require continuous critical re-evaluation. For this reason, the aim of this work was analysis of the feasibility of this type of treatment, with the categorization of indications, are essential for understanding the place and role of TPE in the treatment of these patients group.
Methods: Retrospective analysis of indications for the total of 1,075 TPE during the period from 2011 to 2016, as well as categorization according to criteria AABB at the Blood Transfusion Institute Nis, Serbia, has been done. TPEs are performed using the blood cell separator Haemonetics MCS+, according to the applicable standards and recommendations about the number and frequency of procedures, optimized time of application and the amount of extracted plasma.
Results: All of 1,075 TPE procedures are analysed and categorized, and the following results were obtained, category I was represented in 44.2%, 31.8% category II, category II –III in 20.2% and category III in 7.8%, while category IV was not represented. The most common indication was myasthenia gravis (MG) 55 patients, 205 TPE, followed by Guillain – Barre syndrome with 34 patients, 141 TPE, multiple sclerosis 29 patients, 109 TPE, hyperbilirubinemia 21 patients, 72 TPE, CIDP 13 patients, 49 TPE, and thrombotic thrombocytopenic purpura (TTP) was represented in 8 patients, 35 TPE, and other in I, II, III category.
Summary/Conclusions: The results suggest that the indications for TPE predominantly belong to category I and II, while disorders in the category IV were not presented. This can be interpreted as rationalization and optimization of the TPE application, as a special form/modality of the treatment. The positive therapeutic effect of TPE in the treatment of patients depended upon the nature of the basic disease, its stage, general condition, volume of plasma removed and additional therapy.

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TREATMENT OF CHRONIC INFLAMMATORY DEMYELINATING POLYRADICULONEUROPATHY (CIPD) WITH PLASMA-EXCHANGE: SHORT-TERM RESPONSE CASE REPORT

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Background: Chronic Inflammatory Demyelinating Polyneuropathies (CIDP) are neurological diseases characterized by demyelination of peripheral nerves with mononuclear cell infiltrates, electrical conduction slowing or block and elevated cerebrospinal fluid protein with no cells. Numerous data of literature have suggested that these illness show an immune mediated pathogenesis. The clinical picture is characterized by progressive or relapsing symmetrical motor or sensory symptoms and signs in more than one limb. Therapeutic strategies are represented by prednisone, immune suppressive treatment, as well as plasmapheresis and intravenous immunoglobulins at high doses (IVIg-HD), singly used and/or at combination. The role of the Plasma-Exchange (TPE) is correlated with published evidence and indicates that it remove from the blood circulation the mediators of the inflammatory (antibodies, cytokines, growth factors) before they can activate the mechanism of the damage
Aims: In this study, we report a case of a 50-year-old woman affected by CIPD and treated from the 2014 with periodic plasma exchange according to our guidelines
Methods: The procedures of TPE were performed with the cell separator COM-TEC (FRESENIUS). It was executed 1 exchange of plasmatic volume/session, using as liquid of substitution an albumin solution 4%.
Results: A 50-year-old woman was admitted to the intensive care unit of Monaldi Hospital in the 2014 following severe difficulty to breathe and to walk. These symptoms are not regressed with prednisone and immune suppressive treatment. We have begin the procedures of TPE as reported in the methods. The patient responded to a cycle of TPE of 3 sessions/week for 2 weeks and then 1/month for 1 year. Following such treatment the patient showed a complete remission of the symptoms. After 2 year, it was observed a disease relapse, therefore it was necessary to execute a new TPE therapy accompanied by the intravenous IgG high-doses. Such therapy was periodically continued every 3 months during the following years. At present, the patient is periodically (every 3–6 months) undergone to TPE treatment to prevent the relapse of the disease symptoms
Summary/Conclusions: Evidence from trials suggests that plasma exchange provides significant short-term benefit in the chronic inflammatory demyelinating polyradiculoneuropathy (CIPD). Numerous patient respond to repeated administrations of IVIg high doses/TPE to achieve a complete disease relapse. Here we show that the periodically TPE therapy is able to provide long-term benefit of the disease symptoms and to prevent the illness.

P-608

RELAPSED NEUROMYELITIS OPTICA SPECTRUM DISORDER, SUCCESSFULLY TREATED BY THERAPEUTIC PLASMA EXCHANGE: A CASE REPORT

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Background: Neuromyelitis optica (NMO) and NMO spectrum disorder (NMOSD) are debilitating inflammatory disease of the central nervous system, which mainly affects the spinal cord and optic nerves. NMOSD often presents as a relapsing course, accompanied by acute exacerbations frequently.
Aims: When intravenous corticosteroid pulse therapy is ineffective in acute attack, therapeutic plasma exchange (TPE) can be implemented as an effective rescue therapy.
Methods: Here, we present the case of a 40-year-old woman who had a second attack of NMOSD despite oral immunosuppressant therapy.
Results: The patient presented paresthesia and pain from the nipple to the thigh, which developed one day before she visited the hospital. Spine magnetic resonance imaging revealed T3~T8 involvement with signal enhancement at the T5–T7 level, which correlated with the symptom of the patient. Her symptom deteriorated, and paraplegia developed despite corticosteroid pulse therapy (methylprednisolone 500 mg intravenously, once a day) for five days. Cerebrospinal fluid (CSF) study revealed increased IgG level to 9.02 mg/dl (normal range: 0.63–3.35 mg/dl) and total protein level to 57.2 mg/dl (normal range: 15–45 mg/dl). The result of the NMO-IgG/Aquaporin 4 antibody test with indirect immunofluorescence was moderately positive. The patient was diagnosed as having acute attack of NMOSD, which was resistant to corticosteroid therapy, and received five series of therapeutic plasma exchange (TPE). Single volume TPE was performed daily with replacement fluid containing 5% albumin. Her symptoms improved early in the course of TPE and she could walk again. On the seventh day, she was discharged with much improved symptoms.
Summary/Conclusions: NMO/NMOSD rarely has a progressive course, and neurological deficit solely results from incomplete recovery from each attack. Patients can develop lifelong disabilities, which can occur even after just one exacerbation. Thus, early diagnosis of the disease and prompt initiation of TPE combined with high-dose corticosteroid therapy is crucial for patients' long term outcome.

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A CASE REPORT: THE EFFICACY OF THERAPEUTIC PLASMA EXCHANGE IN THE MANAGEMENT OF A PATIENT WITH ATYPICAL PRESENTATION OF THROMBOTIC THROMBOCYTOPENIC PURPURA

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Background: Thrombotic thrombocytopenic purpura (TTP) is typically characterized by a pentad of thrombocytopenia, microangiopathic hemolytic anemia (MAHA), fever, neurological abnormalities and renal dysfunction. This a rare antibody mediated disorder characterized with severe deficiency of ADAMTS13 activity due to autoantibodies against the protein. Therapeutic Plasma Exchange is considered as the first line of management as per the current ASFA guidelines and has help transform this potentially fatal disease into a curable one.

Aims: There are many atypical presentations of TTP as a result of which treatment is delayed. Here we describe a case of atypical TTP and the challenges faced in treating this patient. It is important to manage TTP in a timely manner without any delay, as it will affect the prognosis.

Methods: Patient was a 24-year-old female who presented to the Emergency department with faintishness and Shortness of breath. Initial investigations revealed high capillary blood sugar, positive urine ketone bodies, severe metabolic acidosis and hypokalemia, hemoglobin of 10.5 g/dl. The patient was initially managed as diabetic ketoacidosis. Initial blood picture showed reactive changes due to diabetic ketoacidosis. The history was not consistent. Despite rigorous management the patient persisted with metabolic acidosis, deteriorating with high blood pressure and drowsiness. She developed acute renal failure as such hemodialysis was carried out during which she went into cardiac arrest. She was resuscitated and sent to the intensive care unit. .

It was observed that the patient had spontaneous bleeding from the nose and cannula sites, muscle weakness, and generalized tonic-clonic seizures. Due to poor respiratory effort the patient was given assisted ventilatory support.

The following parameters were observed: anemia, neutrophil leukocytosis, elevated CRP, impaired clotting with high APTT (53.0s), deranged renal function tests, mild thrombocytopenia (112×10^9/l), total bilirubin 0.36 mg/dl, LDH (595U/l). Repeat blood picture showed a few red cell fragments with medium low platelets and neutrophil leukocytosis and together with neurological and renal changes suggestive of TTP. Potential diagnosis of TTP was made. Cryo poor plasma (CPP) was started at 15 ml/kg and steroid treatment was initiated.

Prior to starting plasma exchange the following challenges were encountered. A femoral line was not possible due to the persistent groin abscess as such a jugular venous access was gained. The patient was unconscious during this time with low GCS. Patient was hypertensive and severely edematous. Daily one volume plasmapheresis was done keeping a negative fluid balance using CPP as the replacement fluid.

Results: The patient showed signs of improvement while the TPE was going on. The platelet count returned to normal in 3 days. TPE was carried out for three more days.

Summary/Conclusions: Many cases of TTP do not present with the classical pentad and may come with minimal features of MAHA and variable thrombocytopenia. Clinical suspicion is highly important to do timely intervention to manage TTP patients. Without treatment mortality rates reach 90%. This shows that even atypical cases of TTP can be managed effectively with therapeutic plasmapheresis using a multidisciplinary team approach. TTP can present with an atypical presentation and if suspected the probable diagnosis should be made without any delay.

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THERAPEUTIC APHERESIS – A CENTER EXPERIENCE

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Background: Therapeutic Apheresis (TA) is a procedure consisting of the removal of a pathogenic substance from a patient's blood by an extracorporeal medical device.

The component substitution solution is determined by the disease to be treated, and the effectiveness of the procedure depends on the patient's blood concentration, processed volume and the equilibrium between blood and the extravascular fluid distribution.

The actual guidelines for using TA, in a wide variety of diseases are increasing, as primary or adjunct therapy

Aims: Evaluate the activity of Therapeutic Apheresis procedures in the last 6 years, in our Center.

Methods: Retrospective review of all TA performed from 2011 to 2016. 368 procedures were analyzed in 25 patients. All the procedures were done by continuous flow method using the COBE Spectra system®. The indications, clinical results and technical factors are discussed.

Results: 18 patients were submitted to Therapeutic Plasma Exchange (TPE) due to thrombotic thrombocytopenic purpura (TTP) and haemolytic uremic syndrome (1). In 4 patients a possible trigger was suggested: one patient after *Escherichia coli* intestinal infection, another after *pneumococcal* vaccination and 2 patients after pregnancy (one congenital and the other acquired TTP). The other cases were considered idiopathic. TPE was performed daily using inactivated human plasma as replacement solution exchanging 1–1.5 total plasma volume, until the platelet count was above 150×10^9/l and lactate dehydrogenase near normal for 2–3 consecutive days. In all these patients corticosteroids were used as an adjunct therapy at 1 mg/kg/day. Rituximab was also used in refractory or relapsing TTP cases. 3 patients had Waldenstrom's macroglobulinemia with IgM levels >30 g/l and hyperviscosity symptoms. Daily procedures exchanged one plasma volume with a Human albumin 5% solution. The clinical response was considered excellent, except in one patient with deterioration of the clinical condition due to preexisting illness. TPE was also performed in 4 patients with demyelinating disease (3 multiple sclerosis and 1 neuromyelitis optica (NMO)) unresponsive to steroids, as a second-line therapeutic option, associated to immunosuppressive medication and immunomodulation. Initially daily and afterwards every other day procedures as deemed necessary (e.g.: in NMO), one plasma volume was exchanged with a human albumin 5% solution with satisfactory clinical and imagiological outcomes. The implementation of RBCs exchange was initiated in 2015: 3 procedures in 2 Sickle Cell Disease patients (one *homozygotic (SS)* and another heterozygous compound (SC)) with HbS >30%, to prevent secondary stoke and to treat chronic debilitating pain.

Summary/Conclusions: The analysis of TA in our department shows that the procedure was secure. Although hypotension and fluid-electrolyte imbalance may occur, most of these problems were readily reversed. Nowadays in our Centre TA is performed in the field of Haematology and Neurology mostly, but it could play a significant role in the patient therapeutic strategy, in an extensive variety of diseases. Using an evidence-based approach is the best way to standardize care and to provide a platform for innovation to move the field forward. To achieve this, we encourage the sharing of the apheresis centres' experiences so as to reinforce and improve evidence levels and provide a knowledge base to update existing guidelines.

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Abstract has been withdrawn.

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ROLE OF AUTOMATED THERAPEUTIC RED CELL EXCHANGE IN THE SETTING OF ACUTE METHEMOGLOBINEMIA – OUR EXPERIENCE

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Background: Methemoglobinemia which is an altered state of hemoglobin resulting in impaired oxygen delivery to the tissues can be congenital or following exposure / ingestion of various oxidant drugs or toxins. One of the earliest signs of methemoglobinemia is generalized cyanosis not improved on supplementation of oxygen & presence of normal PO2 on ABG. Here we report two cases of acquired Methemoglobinemia following Poisoning in two different patients at two different occasions along with our experience of managing them with automated therapeutic red cell exchange

Aims: To evaluate the therapy outcomes as a first line modality in terms of efficacy & safety.

Methods: Automated therapeutic Red cell exchange on COM Tec (Fresenius) Apheresis system using PL1 kit.

Results: Among the two cases of poisoning, the 2 year old baby aniline dye poisoning with initial Met Hb 24% & Spo2 76% was discharged after 7 days of inpatient treatment & the other case of Nitrate/nitrite poisoning with initial Met Hb 67%& Spo2 86% went LAMA. The Post procedure Met Hb levels & SpO_2 levels of the First & second case were 16%/94% & 24%/93% respectively after one procedure only. The Baby' s symptoms improved within 12–18 h & was off the ventilator in less than 3 days. Meanwhile, immediately following the procedure he was administered

3 doses of Inj methylene blue. The adult patient also underwent a single procedure following which he had dramatic recovery in 6–8 h & was off ventilator in less than 24 h. He was also given one dose of methylene blue.
Summary/Conclusions: Although for drug induced or poisoning related cases, methylene blue is considered as the first line therapy, but it has its own pros & cons in terms of availability, G6PD status, dose, duration, side effects including re methemoglobinemia. Manual red cell exchange has been tried in a few cases with promising results in cases resistant to methylene Blue. Therefore, Automated Therapeutic red cell exchange which is much safe, efficacious & promising treatment modality with lots of potential can be utilized in such cases & which will assure prompt relief following a single or may be a 2 day sitting. Further studies on such cases are required to evaluate the therapy outcomes as a first line modality in terms of efficacy & safety.

P-613

PATIENTS WITH SICKLE CELL DISEASE TREATED WITH RED BLOOD CELLS EXCHANGE: THE EXPERIENCE OF RAGUSA SIMT

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Background: Sickle cell disease (SCD) is a genetic haemoglobin disorder which can cause severe pain, significant end-organ damage, pulmonary complications and premature death.
Aims: We follow 98 patients (pts) (76 pts HbS S β-Thalassemia and 22 pts HbS SS). Sixty-two pts are symptomatic and treated with hydroxyurea (18 pts) or transfusion therapy (50 pts). We have evaluated the efficacy of automatic erythrocytes exchange (AEE) in reducing HbS level on those pts treated with this procedure (43 pts).
Methods: Of 43 pts treated with AEE, we had evaluated Hemoglobin, Hematocrit and HbS values pre- and post-AEE just for 22 pts at the moment. AEE was performed with the Optia Separator (Cobe Spectra) and a target value of 30% usually has been set for Hct post-AEE. We studied the % of reduction of HbS after AEE, on the entire group and on two subgroups. As % of reduction we mean the difference between HbS% average post-AEE and that one pre-AEE. These two subgroups have been divided on the basis of HbS % average of the entire group before AEE (52%; group 1: HbS < 52%, 12 pts; group 2: HbS >52%, 10 pts) to evaluate potential difference in HbS reduction between the two groups by the Wilcoxon test.
Results: An average reduction of 29% (min 19.2%, max 36.5%, mediane 30%, DS ± 4.7%) was observed on all 22 pts. Skewness coefficient was −0.51 and Kurtosis coefficient −0.51, showing an asymmetry of data. For this reason, the two subgroups were studied and a non-significant difference was observed in HbS average reduction, 27.3% in group 1 and 31.51% in group 2.
Summary/Conclusions: The HbS level pre-AEE does not seem to correlate with the % of reduction post-AEE. The difference between the two subgroups evaluated might become significant by studying more patients. Then, a longer follow up could allow us to better evaluate HbS % trend over time (pre and post-AEE) and to establish the more appropriate transfusion rate for each patient.

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POLYGLOBULIA – VENESECTION AND DOUBLE RED CELL APHERESIS TREATMENT

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Background: A disease state with high level of haemoglobin and haematocrit is generally called as polycythaemia. Polyglobulia, or erythrocytosis, means separating increasing in the total red cell mass. The common risk of all types of polyglobulia is associated with the consequences of blood hyperviscosity and the risk of thrombotic complications. The increased hematocrit leads to substantial changes in hemodynamic properties of blood, particularly to increased viscosity, intensified red blood cell aggregability, formation of rouleaux and slow-down of blood flow in the peripheral microcirculation.
Aims: The efficiency and suitability of two cytodepletion methods for treatment of polyglobulia – venesection and double red cell apheresis – were evaluated by a retrospective analysis.
Methods: This study evaluated a treatment of 44 patients (33 male, 11 female). Total no. of procedures = 364, no. of double erythrocytapheresis = 284 (median age = 64.4 years), no. of venesection in series = 80 with an average of 2.13 venesections in 1 series (median age = 69 years). The most frequent diagnosis was polycythaemia vera (n = 26), followed by secondary polycythaemia (n = 17) and 1 patient with familiar erythrocytosis. Treatment at polycythaemia vera and familiar polycythaemia was indicated at Ht > 0.50, with a therapeutic goal of Ht < 0.45. Treatment at secondary polycythaemia was indicated at Ht >0.55, achieved Ht value could not drop below 0.50 or 0.55, respectively. In most cases, is indicated double erythrocytapheresis by Haemonetics MCS+, venesection occurred mainly with patients with a poor venous access or with a bad toleration of apheresis.
Results: Median of collected blood during a single series of venesection = 914.6 ml. Median of initial Ht = 0.54, achieved Ht = 0.50, decrease of Ht = 0.048. Median frequency = 112 days, median no. of venesections = 7/year. At double erythrocytapheresis median of collected RBCs = 415 ml. Median initial Ht = 0.54, achieved Ht = 0.47, decrease of Ht = 0.07. Median frequency = 98 days, median procedures = 3.7 year.
Summary/Conclusions: Double red cell apheresis at most of patients with polycythaemia vera as well as secondary polycythaemia is sufficient for achievement of Ht goal values. For majority of patients, the method is more comfortable than venesection, brings a faster relief and it decreases the number of hospital visits. Double red cell apheresis proves to be a suitable cytodepletion method that quickly and securely corrects the increased hematocrit values at an acceptable cost.

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ERYTHROCYTAPHERESIS AND PHLEBOTOMY TO TREAT RED BLOOD CELL (RBC) DISORDERS: REVIEW FROM THE CELLULAR THERAPY AND APHERESIS UNIT OF COIMBRA HOSPITAL AND UNIVERSITY CENTER (CHUC), 2006 TO 2016

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Background: Therapeutic erythrocytapheresis is a procedure used to remove RBC in patients that present medical conditions in whom the RBC mass has increased, whether they're primary or secondary. In CHUC, erythrocytapheresis has become a routine procedure in the cellular therapy and apheresis unit since 2006, taking over a lot of therapeutic phlebotomies and performed on a daily basis on numerous patients. In CHUC, the majority conditions treated by erythrocytapheresis are hemochromatosis, hereditary and acquired, polycythemia, primary and secondary, and porphyria cutanea tarda.
Aims: This study intends to analyze all the erythrocytapheresis procedures that have been performed in the cellular therapy and apheresis unit from 2006 to 2016, characterize the patients and their RBC disorders, roughly compare between initial phlebotomies that ultimate were replaced by erythrocytapheresis as well as compare the two automated systems that were used in the apheresis procedure.
Methods: A retrospective review of all the erythrocytapheresis procedures performed in the cellular therapy and apheresis unit in an eleven year period (2006–2016) was carried out. Two automated systems performed the apheresis treatments: Com.Tec (CT) by Fresenius Kabi and most recently Spectra Optia (SO) by Terumo BCT. The following parameters were analyzed: gender, age, disorder and genetic mutations of each patient diagnosed with hemochromatosis (H); haemoglobin (Hb), haematocrit (Htc) and ferritin (F) before treatment; removed blood volume and automated system used. All data were treated in Microsoft Excel.
Results: A total of 168 patients (139 men, 29 women) were treated using 2 systems (CT and SO) and also performed phlebotomy during shorter or larger periods. A total of 2,054 procedures were performed, 1,162 (56.6%) in CT, 558 (27.2%) in SO and the remaining were phlebotomies. The average age of patients was 60.2 years (min:16; max:85). The most frequent RBC disorders were H (45.2%) and Polycythemia Rubra Vera (PRV) (20.1%). The most frequent mutation in H was Heterozygous HFE H63D/Normal HFE C282Y (25%), followed by Homozygous HFE H63D/Normal HFE C282Y (17.1%) and Heterozygous HFE H63D/Heterozygous HFE C282Y (15.8%). Average Hb 16 g/dl (min: 12; max:22); average Htc 47.5% (max: 69.5; min: 33.8); average F 232.4 μg/l (max: 6,100; min:4); average extracted volume in SO was 370.6 ml (min: 157; max: 627), in CT 446.2 ml (min: 82; max: 1,289) and in phlebotomy 441.0 ml (min: 20; max: 550).
Summary/Conclusions: It is evident from analyzing the results that the smaller extracted volume is obtained from SO equipment, when compared to CT and phlebotomy. Both apheresis procedures are safe for patients. Because there are few patients who were submitted to erythrocytapheresis using only one of the two systems, it is difficult to evaluate the efficiency in iron removal and to recommend the use of one instead of the other. However, it seems, from the data

available, that SO can do better, but further data analysis is needed. Also, it is crucial that the patients included on a subsequent study follow rigorous criteria of evaluation.

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A MODEL ESTABLISHMENT OF WHOLE BLOOD RETURN-TRANSFUSION AND EFFECT OF RETURN-TRANSFUSED BLOOD TREATED BY RIBOFLAVIN PHOTOCHEMICAL TECHNIQUE *IN VIVO*

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Background: Transfusion therapies including cell therapy and plasma exchange gradually developed and applied in many fields of clinical therapy. Auto-transfusion had been became a worthy sort of blood transfusion in order to save blood resources. However, the mode of transfusion therapy by collecting whole blood, treating whole blood using blood treatment technique *in vitro* and return-transfusing into body itself in time with expect to decrease activity of certain substances to control or cure relative disease haven't been concerned.

Aims: To establish an animal model of return-transfused blood with treatment *in vitro* and to investigate the effect of return-transferred blood treated by riboflavin photochemical technique on body's ability of cytokines secretion *in vivo*.

Methods: Rats with appropriate 300 g weight were selected as experimental animal. Of the blood volume of rat, 10 percent with appropriate 2 ml was collected by orbital venous blood sampling method. The whole blood was treated by riboflavin photochemical technique with 80 μmol/l riboflavin final concentration and 3.6 J ultraviolet dose *in vitro*. In 30 min, the blood with treatment was return-transfused into rats themselves via tail vein. Rat blood was sampled for detection in 0 and 48 h respectively. The main detection indictors were cytokines IL-6 and IL-10. The tests were repeated three times. SPSS 16.0 using *t*-test was used to analysis data. Significant was set at $P < 0.05$ in all cases.

Results: All 20 model rats were survived for at least 48 h after blood return-transfusion in time. The level of IL-6 and IL-10 decreased after return-transfused blood with the treatment by riboflavin photochemical technique. The level of IL-6 was 35.92 ± 9.11 pg/ml in 48 h compared with 39.75 ± 6.86 pg/ml in 0 h. The level of IL-10 was 46.03 ± 9.17 pg/ml in 48 h compared with 56.95 ± 12.55 pg/ml in 0 day and there were statistical differences $P < 0.05$.

Summary/Conclusions: Successful model establishment of blood return-transfusion was contributed to an approach of investigating blood transfusion therapy in the future. Return-transfused blood which was treated by riboflavin photochemical technique effectively impaired the body's ability to secrete cytokines, especially IL-10, *in vivo*.

Evidence based transfusion medicine practice

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PATIENT BLOOD MANAGEMENT IN PATIENTS UNDERGOING ARTHROPLASTY FOR FRACTURED NECK OF FEMUR – RESULTS FROM A UK NATIONAL COMPARATIVE AUDIT IN 2015

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Background: Patient blood management (PBM) involves the application of best evidence to optimise the care of patients who may require transfusion. A UK national audit of PBM interventions was performed among a range of surgical procedures where transfusion is common. Whilst not feasible to undertake pre-operative optimisation of haemoglobin prior to surgery for fractured neck of femur, we wanted to assess implementation of other PBM measures in this relatively urgent setting.

Aims: To determine clinical practice around PBM interventions in patients undergoing arthroplasty for fractured neck of femur.

Methods: UK Hospitals were asked to collect data prospectively on consecutive transfusion recipients undergoing surgery over a 3-month period in 2015. Standards were based on the NICE transfusion guidelines and anaemia was classified based on WHO criteria.

Results: Data were received from 113 sites on 1,044 patients who had undergone arthroplasty for fractured neck of femur. 74% were female and the median age was 86 years (IQR 80–91). Pre-operative haemoglobin (Hb) results were available in 1,006 patients (97% available within 2 days of surgery) and the median Hb was 108 g/l (IQR 98–120). Of the patients with Hb documented, 538 (73%) women and 230 (89%) men were anaemic. Ferritin was checked in 46 (14%) of anaemic patients; 18 (39%) had a ferritin of less than 100, of whom 2 (11%) received supplemental iron. 127 (12%) were transfused preoperatively, 180 (17%) intraoperatively and 827 (80%) postoperatively. The median number of units transfused per episode was 2 units. Tranexamic acid was administered in 120 cases (11%) and cell salvage used in 6 patients (0.6%). Among postoperative transfusion recipients, Hb levels were available for 808 patients, of whom 104 (13%) were transfused according to a restrictive threshold and 186 (23%) received a single unit before a further Hb check. 172 (16%) patients received oral iron and 2 (0.1%) intravenous iron, postoperatively. In-hospital mortality was 87 (8%) and among discharged patients the median Hb checked within 3 days was 104 g/l (IQR 96–112).

Summary/Conclusions: Among this representative cohort of UK hip fracture patients undergoing surgery, evidence-based PBM interventions were frequently omitted. Anaemia was common but was rarely investigated fully. There was little evidence of adherence to a restrictive transfusion strategy or a single unit policy and there was minimal use of tranexamic acid or cell salvage. NHS Blood and Transplant are working with hospitals to improve the implementation of PBM.

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COMPARISON OF THE EFFICACY OF Γ-IRRADIATED PLATELET CONCENTRATES (PCS) SUSPENDED IN 100% PLASMA OR 30% PLASMA AND 70% PLATELET ADDITIVE SOLUTION (PAS)

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Background: The application of PAS may enhance infection and immunological safety of the PCs. Moreover, biochemical parameters of PCs suspended in PAS and plasma (PCpas) are more stable than in PCs suspended only in plasma (PCp). Whereas leucofiltration does not remove all leucocytes from PCS, γ –irradiation of PCs minimizes the risk of transfusion-associated graft vs host disease at haematological patients. The impact of γ–irradiation on the efficacy of PCpas vs PCp is unknown.

Aims: to comparison the efficacy of transfusion of γ–irradiated PCp with γ–irradiated PCpas.

Methods: We evaluated the efficacy of 50 PC transfusions in thrombocytopenic patients (25 transfusions of PCp suspended in 100% plasma and 25 transfusions of PCpas suspended in 30% plasma plus 70% of PAS). All PCs were γ–irradiated (25 Gy) and leucoreduced. The median PCs collection target was 5*1011 platelets. The efficacy of PC transfusions was assessed by TEG (TEG 500, Haemoscope) and Corrected Count Increment (CCI) for platelets. For TEG Peripheral venous blood samples were collected into tubes containing sodium citrate. A volume of 340 μl of citrated patient blood was loaded into each TEG cup. A volume of 20 μl of 0.2 M

CaCl2 was added to re-calcify the samples. Analysed TEG parameters were maximum amplitude (MA) before (MA0) and 1 h after (MA1) PC transfusion. For exception of the influence on MA1 of fibrinogen changes, we assessed MA of functional fibrinogen before (MAFF0) and 1 h after the transfusion (MAFF1). CCIs were assessed 1 h after (CCI1) and 24 h after (CCI24) the PC transfusion. Criteria of effective PC transfusion were CCI1 > 7.5; CCI24 > 4.5; MA1 > 44 mm.

Results: According to the CCI1 and CCI24 criteria, the effective transfusions were obtained in 96% and 76% of cases in the PCP group and in 100% and 72% in the PCPAS group, respectively. There were no significant differences between the groups. The MA1 was significantly higher than MA0 as well in PCP group (39 ± 3.5 mm vs 70 mm±1.8, P = 0.0001) as in PCPAS group (35.8 ± 4.8 mm vs 60 ± 3.2 mm, P = 0.0005). There were no significant differences in MA change between the groups. The MAFF0 and the MAFF1 did not differ significantly in the PCP group and in the PCPAS group (23.4 ± 1.4 mm vs 27 ± 2.2 mm, P = 0.18 and 27.5 ± 2.5 mm vs 28.3 ± 2.2 mm, P = 0.8, respectively). Thus, the increase of MA after PC transfusion was associated with platelet function and quantity increment, but not with fibrinogen level changes.

Summary/Conclusions: In thrombocytopenic patients the γ-irradiated PCs suspended in 100% plasma as effective as PCs suspended in PAS and plasma.

P-619

MORTALITY AFTER BLOOD TRANSFUSION FROM DONORS WITH A HISTORY OF PREGNANCY

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Background: Transfusion of red blood cells from female donors has been associated with decreased survival of male recipients. The underlying mechanism is unknown but could be related to pregnancy-induced changes in the blood of female donors.

Aims: To quantify the association between recipient survival and red blood cell transfusion from female donors with and without a history of pregnancy.

Methods: We performed a cohort study of all first time transfusion recipients during a 10-year period at six major Dutch hospitals. Primary analyses were performed on the "no-mixture" cohort: either all transfusions from male donors, or all from female donors without a history of pregnancy, or all from female donors with a history of pregnancy. Additional analyses were also performed on the full cohort. The association between mortality and exposure to transfusions from ever-pregnant or never-pregnant female donors was analyzed using life tables and time-varying Cox proportional hazards models.

Results: The cohort for the primary analyses consisted of 24,424 patients who received 46,117 red blood cell transfusions. The full cohort included 60,912 patients who received 230,099 transfusions. Among male recipients of a single transfusion from a male donor the cumulative incidence of death was 20.4%, this was 22.3% after a transfusion from a never-pregnant female donor (difference: 1.9% (CI: −3.0% to 6.7%)) and 25.4% after a transfusion from an ever-pregnant female donor (difference: 5.0% (CI: 0.3–9.8%). Among female recipients of a single transfusion from a male donor the cumulative incidence of death was 20.4%, this was 20.8% after a transfusion from a never-pregnant female donor (difference: 1.1% (CI: −3.6% to 5.8%)) and 22.3% after a transfusion from an ever-pregnant female donor (difference: 2.7% (CI: −2.0% to 7.3%)). The hazard ratio for death after one additional unit of red blood cells from a never-pregnant female donor, compared to a unit from a male donor, was 0.968 (CI: 0.848–1.104) for male patients and 1.092 (CI: 0.961–1.241) for female patients. The corresponding hazard ratio after transfusion from an ever-pregnant female donor was 1.152 (CI: 1.033–1.286) for male patients and 1.038 (CI 0.911–1.183) for female patients. For male patients this corresponds to an attributable risk of 24%. By stratifying according to recipient age male recipients under 50 years of age had the highest hazard ratios for death after transfusion of red blood cells from ever-pregnant female donors. The hazard ratios of 1.778 (CI: 1.110–2.847) and 1.601 (CI: 1.031–2.486) in male patients aged 0–18 and 18–50 years of age, corresponded to an attributable risk of 57% and 64%, respectively. Analyses of the full cohort yielded similar results.

Summary/Conclusions: Our results confirm the association of red blood cell transfusions from female donors with decreased survival of male recipients, but also limit this association to female donors with a history of pregnancy and male recipients under the age of 50. This opens new avenues for further mechanistic research and, in the future, might lead to changes in transfusion strategy.

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IMPACT OF PROTOCOL- BASED MANAGEMENT USING THROMBOELASTOMETRY ON TRANSFUSION REQUIREMENTS IN CARDIAC SURGERY

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Background: Cardiac surgeries with cardiopulmonary bypass (CPB) are associated with high rate of allogeneic blood transfusion. Because of the complex and multifactorial nature of acquired coagulopathy during CPB, decision for transfusion is often empirical and based on the clinical judgments. However, Thromboelastometry has been introduced as a coagulation point of care test for hemostatic management. .

Aims: In this study, we evaluated impact of using a thromboelastometry-based protocol on transfusion requirements in patients undergoing combined coronary-valve cardiac surgery.

Methods: 80 patients scheduled for elective combined coronary-valve surgery were included in a randomized clinical trial study. Patients were randomly allocated to the Rotem (n = 40) or control groups (n = 40). For all patients in the Rotem group, thromboelastometric tests was performed before and after CPB using Rotem device. Hemostatic management was conducted using blood products and fibrinogen or PCC (prothrombin complex concentrate) according to a defined thromboelastometry – based protocol. In the control group, hemostatic management and transfusion was conducted according to the routine practices including conventional coagulation testing and clinical judgments. Finally, transfusion requirements were compared between groups

Results: Use of thromboelastometry- based protocol resulted in 67% reduction in consumption of cumulative blood products as well as 23% in the percentage of patients transfused. This reduction was especially evident in relation to FFP and platelet consumption. In the Rotem group, the percentage of patients who received FFP or platelet concentrates was significantly lower in comparison to the control group (2.6% vs 31.8% and 2.6% vs 35.9%, respectively, P < 0.001). No significant differences were found in the percentage of patients receiving RBC units (48% in the Rotem group vs 53% in the control group, P > 0.05).

Summary/Conclusions: Considering with known and unknown adverse effects of allogeneic blood transfusion, there is a concern that many transfusions are inappropriate. Having an interventional protocol incorporated thromboelastometry tests results in avoidance of inappropriate transfusion and decrease in allogeneic blood consumption.

P-621

INCREASING PREVALENCE OF ANEMIA AFTER HOSPITAL DISCHARGE AND LONG TERM CLINICAL OUTCOMES

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Background: Anemia has been associated with increased morbidity and mortality. However, multiple randomized clinical trials support decreased red blood cell (RBC) transfusion and tolerance of in-hospital anemia. Long term outcomes related to practice change in the management of anemia have not been described.

Aims: To describe the incidence of moderate and severe anemia (hemoglobin levels less than 10 g/dl) at and following hospital discharge and associated RBC transfusion, re-hospitalization, and mortality events within 6 months of hospital discharge.

Methods: Retrospective, observational study from 2010 to 2014 including 392,202 inpatients (697,685 hospitalizations) who were discharged from 21 hospitals. Hemoglobin levels, RBC transfusion events, re-hospitalization, and mortality within 6 months of hospital discharge. We used χ^2 and Kruskal–Wallis tests to compare annual frequencies and trends. To investigate changes in 6-month mortality following hospital discharge, logistic regression models were used, fitting main effects for age, sex, emergency admission, year of admission, Charlson score, and severity of illness.

Results: From 2010 to 2014, the incidence and prevalence of moderate or severe anemia at hospital discharge increased (23% to 26% and 24% to 29%, respectively; both P < 0.001). In parallel, the proportion of patients in whom anemia had resolved within 6 months of hospital discharge decreased (41% to 33%; P < 0.001). Over the

same time period, incidence of RBC transfusion and re-hospitalization within 6-months of hospital discharge decreased (both $P < 0.001$). While unadjusted 6-month mortality was unchanged ($P = 0.67$), adjusted analysis showed decreased mortality with an odds ratio (OR) of 0.71 (95% CI, 0.67–0.76; $P < 0.001$) in the final as compared to the first year of the study period. In addition, the annual decline in risk-adjusted mortality did not differ between patients with moderate and severe anemia and all other patients (OR 0.93 [95% CI .91-.95] vs OR .90 [95% CI .89-.91]; $P = 0.36$).

Summary/Conclusions: From 2010 to 2014, moderate and severe anemia increased at the time of and within 6 months of hospital discharge. This increase was not accompanied by a rise in subsequent RBC utilization, re-hospitalization, or unadjusted mortality. Declines in risk-adjusted mortality paralleled those of non-anemic patients.

P-622

TRANSFUSIONS OF RED BLOOD CELLS WITH A RARE PHENOTYPE FROM THE SANQUIN BLOOD BANK OF FROZEN BLOOD

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Background: In the Sanquin Bank of Frozen Blood (SBFB), as part of Sanquin Blood Supply, autologous and allogeneic frozen red blood cells (RBCs) with rare phenotypes are stored at −80°C or −196°C. The most important indications for storing those RBCs are patients with allo-antibodies against high frequent blood type antigens (HFA), and patients with a combination of different allo-antibodies or antibodies against very rare blood groups. Also RBCs of patients who developed severe haemolytic transfusion reactions in the past without identified RBC antibodies are stored in the SBFB.

Aims: Because the maintenance of this frozen stock is very costly, we evaluate at a regular base the number of issued units, the indication for ordering these units, and whether these units are used after thawing. We also recorded the adverse events during or after transfusion.

Methods: We have evaluated all cryopreserved and thawed units issued from January 2010 to December 2016. During this episode, an evaluation form was enclosed with all thawed RBCs products with a request for feedback for indication for transfusion, transfusion reactions and the condition of issued units. Analyses included indication for transfusion, number of units transfused (both national and international), number and gender of patients, autologous vs allogeneic units, rare blood group characteristics and adverse events.

Results: During this episode a total of 156 cryopreserved and thawed RBCs were used, 150/156 (96%) in the Netherlands and 6/156 (4%) abroad. These units were issued to 39 patients, of which 32/39 (82%) were female. Transfusion indications for these blood products were haematologic diseases (51%; mainly patients with haemoglobinopathies), pregnancy/delivery (14%), intestinal haemorrhage (8%), orthopaedic surgery (4%), heart surgery (2%), and renal insufficiency (4%). 22/156 (14%) RBC units were ordered precautionary before delivery of which 13/22 (59%) were not transfused. Of all issued units 22/156 (14%) were autologous and 134/156 (86%) were allogeneic stored RBCs. 37/39 (95%) of the patients had allo-antibodies against a high frequent antigen (HFA), while only 2/39(5%) of patients had haemolytic transfusion reactions in the past without identified RBC antibodies. From 120/156 (77%) products, evaluation forms were returned. All units were delivered in good order. No severe (grade 2 or higher) transfusion reactions were reported to Sanquin Blood Supply or to the National Hemovigilance Office in the Netherlands (TRIP).

Summary/Conclusions:

- Most of the cryopreserved and thawed units were used in patients with haematologic diseases (i.e. haemoglobinopathies).
- Most of the patients (95%) had allo-antibodies against a high frequent antigen (HFA).
- Except for obstetric patients, all issued RBC units were used for transfusion.
- The use of autologous units is low 14%, indicating a sufficient allogeneic supply in case of HFA.
- No severe transfusion reactions were reported.

P-623

COMPARING THE NUMBER OF ANTIBIOTIC TREATED INFECTIONS OF PRIMARY AND SECONDARY IMMUNODEFICIENCY DISEASE PATIENTS ON IMMUNOGLOBULIN REPLACEMENT THERAPY – PRELIMINARY FINDINGS IN AN AUSTRALIAN COHORT

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Background: Immunoglobulin replacement therapy (IRT) in the form of intravenous immunoglobulin (IVIg) or subcutaneous immunoglobulin (SCIg) is used to prevent recurrent severe infections seen in patients with primary immunodeficiency (PID) or secondary immunodeficiency disease (SID). While hypogammaglobulinaemia in PID is due to a genetic inability to produce functional Immunoglobulin G (IgG), hypogammaglobulinaemia in SID is secondary to malignancy or drug therapies.

Aims: To compare the number of antibiotic treated infections for Australian adult SID vs PID patients over consecutive years of IVIg then SCIg treatment.

Methods: This was a non-interventional retrospective study on a cohort of 14 PID and 13 SID patients treated at the Sunshine Coast Hospital and Health Service (SCHHS). Data was collected from medical charts at SCHHS and from patient's treating General Practitioners over their last year of IVIg and first year of SCIg treatment. IVIg was administered every 4 weeks and SCIg administered weekly with one quarter of the IVIg dose.

Results: The total number of infections that required antibiotic treatment for the PID cohort over the year of IVIg was 31 infections, and for the year of SCIg treatment period were 25 infections. For the SID cohort there were 24 infections while on IVIg and 29 infections while on SCIg. Upper Respiratory Tract Infections (URTI) were the most frequent infection in both groups. For the PID cohort there were 15 episodes of URTI while on IVIg and 16 while on SCIg. Interestingly, for the SID cohort there were 8 episodes of URTI while on IVIg and 17 while on SCIg. The number of hospitalisations due to infection for PID patients were 4 on IVIg and 2 on SCIg, and for the SID patients 3 on IVIg and 1 on SCIg.

Serum IgG trough levels were not available for all patients. Mean serum IgG levels for PID cohort while on IVIg was 8.8 g/l ($n = 13$) and 9.2 g/l while on SCIg ($n = 9$). In the SID cohort ($n = 12$) the mean serum IgG level was 7.1 g/l on IVIg and 8.2 g/l while on SCIg.

Summary/Conclusions: There was a decrease in number of infections for the PID cohort when they switched from IVIg to SCIg. In contrast, there was an increase in number of infections when the SID cohort switch to SCIg. However, the significance of this finding is limited by the small sample size.

Presently, there is very little published data on SID patients using SCIg, as most reports consist of a larger PID cohort combined with a small SID cohort. The results of this study, raises the question of whether clinical efficacy of switching SID patients from IVIg to SCIg can be based on findings from combined PID/SID cohorts.

Therefore, a study of a larger cohort over a longer period is currently underway to confirm if SID patients do experience more infections while on SCIg, and to determine whether that is due to progression of underlying secondary disease or the different IRT treatment i.e. SCIg.

P-624

RECOMMENDATIONS FOR TRANSFUSION STRATEGY AND ANEMIA TREATMENT IN CHRONIC KIDNEY DISEASE – SYSTEMATIC REVIEW OF CLINICAL PRACTICE GUIDELINES

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Background: Chronic kidney disease (CKD) has an age-dependent prevalence of ca. 10% in the general population. CKD is associated with anemia, because of

diminished production of erythropoietin due to kidney damage, dysfunctional iron metabolism and shortened erythrocyte survival, especially in advanced CKD stages. A small portion of patients with CKD will progress to end stage renal disease (ESRD) and need kidney replacement therapy or transplantation in their lifetime. The application of blood products is to be used with caution in CKD patients, as it may lead to immunization against alloantigens that could complicate kidney transplantation. International clinical practice guidelines (CPG) for management of patients with non-dialysis CKD contain varying recommendations for the management of anemia in this patient group.

Aims: The objective of this systematic review is to provide a comprehensive overview of international CPG recommendations on anemia management and transfusion strategy in adult patients with CKD.

Methods: A systematic review was conducted on evidence based clinical practice guidelines using databases PubMed, international guideline portals and an additional selective search of the World Wide Web, to identify CPG for non-dialysis CKD issued or updated between 2012 and 2016. Two reviewers independently assessed citations according to the selection criteria. Two researchers assessed the methodological quality of eligible guidelines independently, using the Appraisal of Guidelines for Research and Evaluation (AGREE) instrument. Recommendations for management of anemia were extracted.

Results: The literature search yielded 805 citations. Of these records, 792 were excluded after title and abstract review. 60 potentially relevant guidelines were included in the full text review. 18 eligible guidelines met the inclusion criteria. 8 included CPG issued recommendations on diagnosis, monitoring or treatment of anemia, 2 of these CPG specifically had anemia as the guideline topic. Monitoring was mentioned in 5 guidelines, with a frequency of up to 4 times yearly, depending on CKD stage. General guidelines mentioned blood count as a diagnostic tool, whereas the 2 anemia-related guidelines recommended monitoring treatment success with a combination of Hb, reticulocyte count, ferritin or TSAT. The 3 guidelines that mentioned blood transfusion expressly stated that it should be avoided if possible because of alloimmunization. 3 of the 4 guidelines containing recommendations on erythropoietin therapy provided detailed information on indication and erythropoietin resistance, but contraindications were merely mentioned in KDIGO and NICE guidelines on anemia in CKD.

Summary/Conclusions: Although anemia is a known complication of CKD, only half of the CPG contained information on this topic. Several guidelines contained mostly general information on monitoring for anemia, while anemia guidelines contained more specific information on management of anemia in CKD. Most CPG recommended conservative treatment strategies and mentioned individual patient preference, comorbidities and clinical condition as important considerations when deciding on anemia treatment in adult CKD patients, but merely 3 CPG explicitly mentioned restrictive blood transfusion strategy.

P-625

THE NUMBER OF TARGETED HLA CLASS I ALLELES AND ANTIBODY REACTION STRENGTH IS A POOR PREDICTOR OF PLATELET TRANSFUSION OUTCOME IN PLATELET REFRACTORY PATIENTS WITH HLA CLASS I ANTIBODIES

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Background: The optimal strategy to provide platelet transfusions to refractory hematological patients with HLA class I antibodies is debated. In some centers, cross-match techniques are advocated, while other laboratories use HLA matching procedures or matching based on antibody-specificities. How well these methods predict responsiveness to platelets in refractory patients, in isolation or together, is not known.

Aims: To evaluate the response to cross-matched and HLA class I epitope-matched platelet transfusions and to test the predictive power of reaction strength and specificity of the HLA class I antibodies for responsiveness to platelet transfusions in platelet refractory patients with HLA class I antibodies.

Methods: 45 refractory patients (34 women/11 men) with hematological disorders were evaluated. Pre-transfusion and 1-h post-transfusion platelet counts were available for all these transfusions (n = 185). For 142 transfusions, the HLA-type of the patient and the donor was known and the degree of mismatch, expressed as eplets, could be evaluated using the Matchmaker software. HLA class I antibody specificity profiles were analyzed using a Luminex platform.

Results: The average 1-h CCI for all transfusions was 10.9×10^9/l. The mean 1-h CCI for HLA-matched transfusions was 12.6×10^9/l and for cross-matched transfusion 8.3×10^9/l ($P < 0.05$). 70% of HLA-matched transfusions were considered successful (1-h CCI $>7.5 \times 10^9$/l), while the corresponding figures for cross-matched transfusions was 43%. Only 15.5% (n = 22) of the transfusions represented a complete match (Eplet 0), all of which were successful. A surprisingly weak correlation was found between 1-h CCI and the degree of Eplet mismatch for transfusions with Eplet >0. To examine if the predictive power of those transfusions could be improved by taking the HLA antibody profiles of the patients into account, we determined the specificity and reaction intensity of the HLA antibodies in 16 patients (93 transfusions) from our cohort. As expected, transfusions given in the absence of specific HLA class I antibodies were mostly successful; only 2 of 16 (6.2%) of the transfusions failed. When the outcome of HLA-mismatched transfusions in patients carrying specific antibodies were evaluated, a very low correlation coefficient was found ($R_2 = 0.16$), which was only slightly better than the correlation between the 1-h CCI and the Eplet score on the same transfusions ($R_2 = 0.09$).

Summary/Conclusions: HLA epitope-matching was superior to cross-matching with regard to the 1-h CCI and the fraction of successful transfusions. Transfusions with no genetic mismatch (Eplet 0), or transfusions with eplet values >0 in the absence of specific antibodies, were almost all successful. When specific antibodies were present, the number of antibody specificities and their cumulative reaction strength did not provide much additive predictive value for responsiveness, suggesting that host factors other than the HLA antibodies themselves are important in determining the pathogenicity of HLA antibodies.

P-626

QUALITY OF EVIDENCE-BASED GUIDELINES FOR TRANSFUSION OF RED BLOOD CELLS AND PLASMA: A SYSTEMATIC REVIEW

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Background: Uptake of research is recognized to be inconsistent. Clinical practice guidelines have the potential to improve health outcomes, increase consistency of care, increase efficiency, improve quality efficiencies and identify research priorities. An increasing number of transfusion guidelines are available, but there has been limited analysis undertaken to appraise these documents.

Aims: The aim of this systematic review was to identify recent evidence based transfusion guidelines for red cells and plasma, and determine the quality, as assessed by a standard tool: the Appraisal of Guidelines for Research and Evaluation (AGREE II). Other aims were to assess duplication and the consistency of the recommendations between guidelines.

Methods: The protocol for this study was published on PROSPERO. MEDLINE and EMBASE were systematically searched for evidence based guidelines from 2005 to July week 1 2015. Citations were reviewed in duplicate for inclusion. Four to six physicians used the AGREE II instrument to appraise the guidelines using the following items: the clarity of the scope and purpose of the guideline, the representativeness (stakeholder involvement) of the guideline development group the transparency and methodological rigour of development of the guideline, the clarity of presentation, the independence from funding agencies when developing recommendations and overall quality. Each item was scored according to the AGREE II instrument on a scale of one to seven, seven representing the highest score. We calculated the median scores as well as a scaled score as suggested by the AGREE II instrument. The scaled score (%) was calculated as (obtained score – minimum possible score)/(-maximum possible score – minimum possible score). The maximum possible score was calculated as 7(strongly agree) × n (items) × n (appraisers). The minimum possible score was calculated as 1 (strongly disagree) x n (items) x n (appraisers). As part of the AGREE II tool, the reviewers were also tasked with considering whether the guideline should be recommended for use.

Results: Of 5,387 citations, 4,325 records were screened after duplicates removed. 24 studies met inclusion criteria. The results for quality scoring revealed variability among appraisers across all items. Median scores, the median of the scaled score and the interquartile range of the scaled score were as follows: scope and purpose: median score 4, scaled score 59%, IQR (49–74%); stakeholder involvement 3.5, 42%, (33–49%); rigour of development 3, 40%, (18–59%); clarity of presentation 5, 64%, (50–80%); applicability 1, 17%, (10–20%); editorial independence 3.5, 40%, (15–60%). Overall quality ranged from 2 to 6. Thirteen guidelines were recommended with or without modifications, seven guidelines were not recommended and there was no consensus on the remaining four guidelines. Seven guidelines described a process to update their recommendations. The 24 guidelines show both duplication and discrepancies in recommendations.
Summary/Conclusions: Despite the recognition that guidelines have a role to address research uptake, our findings document issues of duplication, lack of rigour in development, and inconsistencies in recommendations for the same topic. Improvements to enhance the process of developing guidelines according to the international AGREE standard is suggested.

P-627

CHALLENGES IN THE APPROPRIATE USE OF IRRADIATED BLOOD COMPONENTS AT A LARGE UK HAEMATO-ONCOLOGY CENTRE

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Background: Transfusion Associated Graft vs Host Disease (TA-GvHD) is an almost universally fatal condition caused by engraftment of donor T lymphocytes in transfused blood in an immunocompromised recipient which can be prevented by irradiation of blood components. The risk of TA- GvHD may be short term in some patient groups until recovery of T cell function but is lifelong in others and the British Committee for Standards in Haematology has made recommendations (2010) for use of irradiated blood components based on these risks. Many laboratory transfusion computer systems lack the flexibility to easily remove irradiated flags and patients may continue to receive irradiated products unnecessarily; clear communication between clinical and laboratory teams is essential.
Aims: This retrospective observational study aimed to assess the indication for and appropriateness of irradiated flags and blood components issued over 1 year from January to December 2016 at St Bartholomew's Hospital, London, UK – a level 3 Haemato-Oncology and stem cell transplant centre.
Methods: All patients with an irradiated flag for whom a Group and Screen sample was tested by the laboratory in 2016 were identified retrospectively. Clinical notes were used to identify the indication and duration for the flag and whether this remained appropriate. For those patients with an inappropriate flag, the number of units of red cells and platelets (unnecessarily irradiated) transfused over the year 2016 was recorded.
Results: In 2016 the laboratory received samples on 430 patients with irradiated flags. In 232 (54%) the indication was lifelong – including ATG for aplastic anaemia (18), fludarabine as chemotherapy (54) or conditioning for allogeneic stem cell transplant (81), bendamustine (31), other drugs (3), allograft recipients with chronic GVHD or ongoing immunosuppression (3), Hodgkin's lymphoma (34) and congenital T cell disorders (1). Temporary indications (181, 42%) comprised autologous stem cell transplants (166), allografts off immunosuppression (4) and stem cell harvests (11). 355 (83%) flags were appropriate. The remaining 17% were inappropriate either because a temporary flag had not been removed (58, 13%) or the flag was never required (17, 4%). Patients with inappropriate flags were issued a total of 849 unnecessarily irradiated components in 2016 – 497 units of red cells and 352 pools of platelets.
Summary/Conclusions: Challenges in removing flags from patients with temporary indications for irradiated blood products can result in continued use unnecessarily. Irradiated red cells have a shortened shelf life (14 compared with the standard 35 days in UK) which can affect laboratory stock control and cause increased wastage. Irradiation of red cells reduces deformability, increases susceptibility to oxidative stress, increases *in vitro* haemolysis and potassium leakage and reduces *in vivo* recovery. Although these changes are felt to be clinically insignificant, there have been few studies comparing outcomes in practice. There is risk of delays in patients receiving transfusions if irradiated products are not readily available. Special requirements flags on laboratory information systems can help ensure that patients receive irradiated components when required but robust systems are also needed for removal of temporary flags when no longer indicated.

P-628

ELEVATED IMMUNOSUPPRESSIVE ACIDIC PROTEIN CORRELATES WITH IMMUNE SUPPRESSION IN PATIENTS RECEIVING ALLOGENIC BLOOD TRANSFUSION

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Background: Allogenic blood transfusion produce generalized immunosuppression in the recipient. This is due to a variety of changes in the immunological functions, including decreased function of natural killer cells, macrophage migration to sites of injury, lymphocyte proliferation, and cutaneous delayed hypersensitivity.
Aims: The broad objective of this study was to determine and compare the levels of pre and post-transfusion immunosuppressive acidic protein with the CD4, CD8 and haematological indices of patients receiving multiple transfusions.
Methods: Institutional ethical approval and informed consent of participants was obtained in this cross sectional study. Participants were trauma patients receiving care in a tertiary hospital facility. 5 ml of pre and post transfusion (48 h) blood samples was obtained from consenting subjects into K3 EDTA and Plain sample bottles after the completion of a structured questionnaire. Blood samples was also obtained from units transfused to the subjects
Results: There was a significant rise ($P < 0.05$) in the mean value of IAP observed after transfusion (712.10 ± 512 μg/l) compare to pre-transfusion values (662 ± 190 μg/l). The observed increases correlated with decline in the CD4 counts from 563 ± 153 cells/μl before transfusion to 315 ± 93 cells/μl after transfusion. There was no significant relationship ($P > 0.05$) between the values of IAP, CD4 and CD8 in transfused units with post transfusion values. The calculated CD4:CD8 ratio was 1.1 before transfusion and 1.4 post transfusion. Apart from the PCV which rose significantly ($P < 0.05$) from 23 ± 6% before transfusion to 28 ± 5% post transfusion, there was no significant difference in the red cell indices, WBC and other parameters.
Summary/Conclusions: The transfusion of allogeneic blood is associated with immunosuppression, which is evidence by changes in the CD4 and CD8 counts and elevated levels of immunosuppressive acidic protein. The rise in levels of post transfusion IAP correlates with a decline in CD4 counts in patients receiving allogeneic blood.

P-629

SICKLE CELL DISEASE: THE ADVANTAGES OF PHENOTYPE COMPATIBLE RED CELLS AND UTILITY OF GENOTYPING IN ERYTHROCYTAPHERESIS

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Background: Sickle cell disease (SCD) remains a public health concern with high morbidity and mortality. Red cell exchange (RCE) transfusion is an effective therapy for both acute and chronic complications of SCD. However, with this comes the risk of alloimmunisation and complications of haemolytic transfusion reactions and challenges in the provision of safe blood. Whilst there are no universally accepted transfusion guidelines for these patients, some institutions provide varying degrees of phenotypically compatible red cell units. The efficacy of this strategy in reducing alloimmunisation, and role of genotyping in this situation remains poorly understood.
Aims: To document the alloimmunisation rate in a single tertiary referral centre for SCD patients undergoing RCE with red cell units that are phenotypically compatible for clinically significant antigens, and to consider the utility of genotyping in the setting.
Methods: A retrospective audit of transfusion laboratory records, pathology results and medical histories was conducted for SCD patients undergoing automated RCE between 1 January 2010 and 30 June 2016. Data on patient blood group and phenotype (by serologic and genotyping methods) and antibodies at time of first presentation to our institution were collected. For each RCE episode, antibody screening results, number of units transfused and information on the effectiveness of the exchange were recorded. Patients received red cell units that were phenotypically compatible for ABO, Rh (c, C, D, e, E), Kell, Kidd and Duffy antigens. Units compatible for MNSs antigens were provided to patients who were serologically phenotyped as Fy(a-b-).
Results: Twenty-eight patients underwent RCE. The median number of exchanges per patient was 51 (range 1–81). The median number of red cell units received per patient was 281 (6–443) and median units per exchange 6 (4–7). The mean HbS percentage post RCE was 23.95%. Fifteen patients (54%) had detectable antibodies or a history of alloimmunisation at their first RCE episode in the study period (median number of antibodies per patient 3, range 1–9). Seven patients developed at least

one new antibody (median 1, range 1–4), 4 of whom had pre-existing antibodies. One of these new antibodies was clinically significant (anti-Cw). Cw was not on the genotyping panel used. Seven patients had historic antibodies that were not detected at the first antibody screen, but were detected in subsequent screens. Two of these were clinically significant antibodies (anti-E and anti-Mur). Genotyping information was available for twenty-six patients. Six patients were found to have the GATA box silencing mutation.

Summary/Conclusions: Our results demonstrate a high alloimmunisation rate in SCD patients. Despite extended phenotype matching of red cell units for RCE, 25% of patients formed additional alloantibodies. Notably, only one was clinically significant. The available genotyping information could not have prevented this. Genotyping was however valuable in extending the donor pool available to those patients who serologically phenotyped as Fy(a-b-) but were found to have a GATA box silencing mutation. The data also highlight the dangers of evanescent antibodies if patients are transfused at other institutions and support calls for national/regional alloantibody databases.

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EVALUATION OF BLOOD ORDERING FOR SURGICAL PATIENTS IN A TERTIARY CARE HOSPITAL IN ZAGREB, CROATIA

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Background: Limited availability and increased demands for blood and blood components, as well as rising costs for testing and wastage impose necessity of avoiding unnecessary transfusions. Blood components, routinely ordered for elective surgical procedures, often remain unutilized and not available for cross-matching while reserved for a specific patient. This may result in the expiry of shelf life and wastage of blood. Various strategies have been developed to reduce the inappropriate use of blood.

Aims: Evaluation of blood ordering for the most common elective surgical procedures at one Clinical Hospital in Zagreb. The purpose of the study is improvement of blood product ordering and utilization and reduction of unnecessary preoperative cross-matching as well as wastage of resources.

Methods: Data were collected from January 2015 till December 2016. Hospital Transfusion Unit received requests for cross-match for patients underwent to the following procedures: orthotopic liver transplantation (OLT), kidney transplantation, total hip replacement, hemicolectomy, Dixon resection, liver resection and nephrectomy. We have calculated for each procedure cross-match to transfusion ratio -C/T (number of units cross-matched /number of units transfused), transfusion probability (number of transfused patients x 100/ number of cross-matched patients) and transfusion index (number of units transfused /number of patients cross-matched). A C/T ratio of >2 and transfusion probability <30% are consider to be indicative of significant blood wastage. TI < 0.5 signifies no need for cross-match.

Results: During the 2-year period, Hospital Transfusion Unit received a total of 888 requests for cross-match and 456 (51.3%) patients were transfused. A total of 7,409 red blood cells (RBCs) units were cross-matched, of which 1,708 (23%) units were transfused. It means that 77% of RBC units were unutilized. The overall C/T ratio for above mentioned elective surgical procedures was 4.33. Considerable variations in C/T ratio were found, depending on the type of the surgery performed. The highest C/T ratio was found for kidney transplant (8.37), followed by nephrectomy (7.66) and Dixon resection (7.49). The lowermost C/T ratio was found for total hip replacement (3.78). The overall transfusion probability and transfusion index were observed as 51.3% and 1.92, respectively. The transfusion index in elective surgical procedures analyzed was above 0.5 in all procedures except nephrectomy (0.47) which indicates the need for cross-match in most procedures from this study. All surgical procedures had the transfusion probability >30% except for Dixon resection and nephrectomy.

Summary/Conclusions: The overall C/T ratio of 4.33 demonstrated that in surgical procedures analyzed, the preoperative assessment of blood requirements led to over-ordering of blood components. However, we are aware that for extensive abdominal surgeries (eg. expanded malignancy diseases) it is difficult to assess blood loss before exploratory laparotomy. We are planning, in collaboration with surgeons and anesthesiologists, to improve the efficacy of ordering and utilization of blood products by defining the maximum surgical blood ordering schedule for these procedures and all other elective surgical procedures in this Clinical Hospital.

P-631

Abstract has been withdrawn.

P-632

LOWER HEMOGLOBIN TRANSFUSION TRIGGER IS ASSOCIATED WITH HIGHER MORTALITY IN PATIENTS HOSPITALIZED WITH PNEUMONIA

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Background: Pneumonia is a significant cause of morbidity and mortality worldwide. Patients with pneumonia requiring hospitalization are generally older, suffer from comorbidities and may need a red blood cell (RBC) transfusion. The hemoglobin (Hb) trigger suggested by the guidelines is 7 g/dl.

Aims: To evaluate the association between the initial transfusion Hb trigger and in-hospital mortality.

Methods: Historical cohort study of consecutive patients hospitalized in internal medicine ward "A" between 2000 and 2014 with pneumonia, who received at least one unit of RBC. Hb trigger was categorized using 7 and 8 g/dl as threshold levels. Data on demography, comorbidities, blood tests and in-hospital mortality were collected. Multivariate logistic regression was used to control for potential confounders.

Results: One hundred males and 77 females were included in the study (median age 80, IQR 71–87 years). The median Hb trigger was 8.10 g/dl (IQR, 7.65–8.60). Patients in the 3 hemoglobin trigger categories (≤7.00, 7–8, >8 g/dl) did not differ for any of the studied variables. In multivariate analysis, only lower Hb trigger ($OR_{\leq 7 vs > 8}$ = 5.24, $OR_{7-8 vs > 8}$ = 2.13, P = 0.035) and higher absolute neutrophil count at admission (P = 0.012) were associated with increased in-hospital mortality.

Summary/Conclusions: In patients hospitalized with pneumonia requiring RBC transfusion, a lower transfusion trigger is associated with increased risk for in hospital mortality.

P-633

RECENT EVIDENCE FAVOURS SPECIFIC GUIDANCE ON TRANSFUSION STRATEGIES IN THE OLDER POPULATION

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Background: The proportion of the population aged 65 and above is growing worldwide. Many diseases relevant to transfusion outcomes increase in prevalence with age. Until recently, little evidence has been available regarding transfusion outcomes in geriatric patients. Restrictive transfusion strategies are increasingly used in healthcare, yet much of the evidence supporting such strategies is drawn from observational studies, and does not focus on the geriatric cohort.

Paediatric patients are recognised as having distinctive transfusion requirements compared to adults due to differences in physiology, and specialist paediatric patient blood management guidelines have been developed. Our study explores the available evidence on transfusion in older patients, to ask whether age-specific blood management guidance is needed for this group.

Aims: This study aimed to identify and review the evidence relating to transfusion outcomes in patients aged 65 years and older. It explored issues of harm vs benefit associated with transfusion, and whether restrictive or liberal transfusion strategies have been shown to be beneficial in older adults.

Methods: A literature review was undertaken using search criteria and strategies pre-defined by the investigators. The review focussed on evidence relating to transfusion outcomes in patients aged 65 years and above. Included evidence was classified as Level I (systematic reviews, meta-analyses), Level II (randomised control trials (RCT)) and Level III (observational) studies.

Results: Ninety-eight articles were included in the analysis. Most papers reported Level III evidence (78%), with fewer examples of Level II (20%), and least of Level I (2%) studies. Several geriatric-relevant papers reporting RCTs were published in

Time after HSCT (days)	30 RBC*	PLT**	90 RBC	PLT	180 RBC	PLT	365 RBC	PLT
GROUP A	3.5 (0–14)	3 (0–23)	4 (0–47)	3 (0–80)	4 (0–75)	3 (0–229)	4 (0–64)	3 (0–100)
GROUP B	6 (2–39)	15 (4–69)	6.5 (2–45)	18.5 (7–67)	6 (2–21)	18 (7–30)	6.5 (2–21)	17 (7–30)
GROUP C	6 (1–25)	9.5 (2–23)	6 (2–14)	8.5 (2–41)	6 (2–22)	8.5 (2–58)	6 (2–26)	8 (2–65)
P	<0.001	<0.001	0.004	<0.001	0.025	0.001	0.072	0.235

*Red blood cell concentrate,
**Platelet concentrate.

2015 and 2016, and thus their evidence is yet to be incorporated into published Level I evidence or guidelines.
Most reports from observational studies (Level III evidence) supported restrictive transfusion strategies, and associated transfusion with poorer outcomes in geriatric patients. By contrast, eight RCTs (Level II evidence) found liberal transfusion strategies provided better outcomes for older patients, and three were equivocal. None of this higher-level evidence supported restrictive transfusion approaches in older patients.
Equivalent or better outcomes for older adults identified by RCTs related to rates of mortality, cardiovascular complications, infections, and post-operative delirium.
Summary/Conclusions: The results of this review indicate that caution should be used regarding restrictive transfusion approaches in older adults, and that liberal transfusion strategies may facilitate better outcomes in this cohort. The results of transfusion studies involving the broader adult population may be misleading in relation to the geriatric cohort.
Further research is needed to explore the reasons older adult patients may be impacted differently by transfusion than the younger cohort. Our findings suggest that specific guidance for transfusion in older adults is warranted in patient blood management guidelines.

P-634

TRANSFUSION REQUIREMENTS OF PATIENTS UNDERGOING ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION FROM DIFFERENT STEM CELL SOURCES AND MODALITIES

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Background: Allogeneic hematopoietic stem cell transplantation (HSCT) is the only therapeutic procedure capable to cure a variety of hematologic disorders. HLA identical sibling donors are the best option for transplantation purpose. For patients without a related HLA-matched donor, umbilical cord blood and related haploidentical provide a potential source of stem cells. Blood product transfusion is critical to support HSCT and blood banks play an important role in managing the patients undergoing HSCT.
Aims: This study investigates the transfusion outcome of patients undergoing HSCT from different stem cell sources and modalities.
Methods: we conducted a retrospective study for analyzing transfusion outcome of patients undergoing HSCT for a 2-year period (2013 and 2014) in a single institution. Transfusion requirements at 30, 90, 180 and 365 days after UCBT were recorded. Platelet (PLT) prophylactic transfusions were administered at a trigger of less than 20,000/μl. Red blood cell (RBC) transfusions were performed for hemoglobin <8 g/dl. Transfusion independence was defined as the day of last transfusion, with no PLT transfusions in the following 7 days or no RBC transfusions in the following 30 days. All products were irradiated before transfusion with 25 Gy. Non-parametric tests were used for comparisons among groups.
Results: The study included 132 patients (median age at time of transplant: 44.1 years, range 9–65), 66 male and 66 female. Sixty-two patients received a related HLA identical peripheral blood HSCT (group A), 40 received an umbilical cord blood transplantation (UCBT) (group B), and 30 received a haploidentical HSCT (group C). Most patients (n = 119) received myeloablative fludarabine based conditioning regimen (MA) and 13 received reduced intensity conditioning regimen (RIC). Transfusion requirements are shown in the attached table. Results are expressed as median and range. When comparing transfusion requirements between group B and C, only platelet transfusion 30 days after transplantation was significantly higher for patients belonging to the group B (P = 0.003). RBC transfusion independence was achieved by 90.3% in the group A, 73.0% in the group B an 76.6% in the group C (P = 0.062), while Platelet transfusion independence was achieved by 91.9% in the group A, 69.2% in the group B and 73.3% in the group C (P = 0.009). With a median follow-up of 587 days (24–1,060), 81 patients (61.3%) are alive.
Summary/Conclusions: UCBT and haploidentical transplantations have significantly higher transfusion requirements than HLA identical HSCT. This information must be taken into account when a HSCT is scheduled.

P-635

THE USE OF RED CELLS IN HOSPICE-BASED PALLIATIVE MEDICINE

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Background: There is known to be variation in the transfusion of red blood cells and guidance advocates appropriate investigations when treating anaemia, consideration of risks, benefits and alternatives and, when transfusing, more restrictive use. This audit of 69% of hospices in the UK looks at physician practice and considers patient safety in terms of being monitored during transfusion.
Aims: - To identify guidelines for good practice in red cell transfusion; To audit medical records of inpatients and day patients transfused in hospices to discover if: Patients are investigated for reversible iron deficiency anaemia; Patients are offered alternatives or adjuncts to transfusion; Patients are weighed as part of managing the risk of TACO; Patients who are not symptomatic are transfused only if their Hb warrants a transfusion; Patients are adequately monitored during their transfusion
Methods: 138/200 (69%) hospices in the UK that confirmed they undertook transfusions invited to enrol in the audit, 82 (59%) of which were able to provide 460 audited episodes. The remainder had no transfusions in that period. Data collection was from October 1st to December 31st 2016, with patients being defined as aged 18 or over and receiving a red blood cell transfusion in a hospice environment. Only a single unit during a transfusion episode was audited, with all other units within that episode recorded as additional units. Sites were sent pre-printed booklets to capture clinical data and data was subsequently submitted online or by returning completed booklets. Data was analysed by NHS Blood and Transplant
Results: 327 patients (71%) were made aware of the transfusion risks, benefits and alternatives. Only 68 (15%) patients were weighed prior to transfusion, 41 of which were below 70 kg, suggesting that some patients may be susceptible to transfusion-associated circulatory overload (TACO). Of 320 (70%) patients with anaemia, 273 (85%) patients had haematinics checked for reversible iron deficiency anaemia, so the remaining 47 (15%) may have been unnecessarily transfused. 145 (32%) patients had a pre-transfusion Hb result of ≥80 g/l, and in the absence of an individualised transfusion threshold these patients may not have needed red cells. 449 patients (98%) did not receive an Hb check after each unit transfused, with 10 (2%) having a check and 1 (<1%) missing. UK guidelines require that all patients receiving more than one unit have the Hb checked between units. 126 (27%) patients received a post-transfusion Hb check. The majority of patients were appropriately observed during their transfusion.
Summary/Conclusions: Risks, benefits or alternatives were not discussed with many patients. Many were not weighed and transfusing low weight patients puts them at risk of TACO. A significant number of patients were not investigated for iron deficiency before transfusion. Transfusion may have been inappropriate in patients whose pre-transfusion Hb was >80 g/l. Most of the patients who received more than one unit of blood did not have a clinical review between units, despite national guidance to do so. The majority did not have a post-transfusion Hb check, suggesting the possibility that the managing physician did not demonstrate that the desired benefit had been achieved. The results from this audit will be fed back to participating hospices and will be used to influence debate on optimum transfusion practice in palliative medicine in UK hospices.

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BONE MARROW IRON OVERLOAD IN TRANSFUSED ACUTE MYELOID LEUKEMIA PATIENTS

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Background: Secondary iron overload due to red blood cell (RBC) transfusions is associated with increased morbidity and mortality in various patient groups. However, attention for secondary iron overload and its side effects in hemato-oncological patients may need improvement. Reasons for this may be the unreliability and/or invasiveness of currently available diagnostic tests to detect iron overload and the possible drawbacks of iron chelation therapy.
Aims: To evaluate the effect of the number of RBC transfusions on bone marrow iron scores.
Methods: This study comprises AML patients in a tertiary treatment center, treated according to current AML treatment regimes. Patients were included when 20 or more units of RBCs were transfused and when bone marrow specimens were available after the 20 RBC transfusion threshold was exceeded. Consecutive bone marrow samples from diagnosis till the end of study (range 2007–2016) were stained with a standardized Perl's staining. The scoring was performed independently by two experienced researchers according to a pre-specified protocol. The slides were blinded to both researchers to prevent bias. Kaplan-Meier survival analysis was performed to assess the median number of RBC transfusions needed to reach the maximum bone marrow iron score. Serum ferritin levels were barely measured in this cohort of patients and could therefore not be related to results of Perl's staining.
Results: In total, 141 bone marrow specimens (median 4 per patient, range 2–8) from 35 patients were included in our study. The mean age was 57.8 years (SD14) and 51% were males. The median number of RBC transfusion received were 34 (range 22–69). Twenty-nine patients (83%) underwent hematopoietic stem cell transplantation. The median number of RBC transfusions to reach a maximum bone marrow iron score was 20 units (range 6–42, IQR 15–26), after a mean of 1.64 chemotherapy courses (SD 0.99).
Summary/Conclusions: RBC transfusion burden in AML patients is associated with high bone marrow iron scores. Bone marrow iron scores may be a valuable indicator of secondary iron overload in transfused AML patients. Whether this may guide iron chelation therapy or phlebotomy needs to be determined.

P-637

LESS CLICKS! INTEGRATION OF THE PRESCRIPTION AND ADMINISTRATION OF BLOOD TRANSFUSION IN THE ELECTRONIC PATIENT FILE

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Background: At the University Hospitals of Leuven an electronic patient file is used where the prescription and administration of medication can be registered in the Electronic Medication Prescription module (EMP). Till now the prescription and scanning of the administration of blood components were not integrated in this EMP. This resulted in additional administrative work for doctors and nurses, as the prescription, order, scanning and administration of blood components were all located in several modules.
Aims: To optimize the prescription and administration of blood transfusion in the EMP.
Methods: In cooperation with the ICT department, the haemovigilance team developed templates for the electronic prescription of blood components in the EMP. Doctors could easily prescribe a blood component, including necessary premedication, and at the same time make the order for the blood bank to deliver the necessary blood component. In the same module nurses could scan the administration of the blood component.
After a pilot test case at the haematology isolation ward, all medical staff were informed by mail and an instruction movie. The implementation at nurse wards has been successful through multiple education sessions.
Results: The reaction of the medical staff was unanimously positive. The templates are easy and quick to use. Only minor changes were made afterwards. The communication by mail was not always sufficient: additional education was given to doctors by the haemovigilance team.
We noticed a better scanning of blood components by nurses. On the pilot ward we observed 99.4% correct administrations on a total of 4.082 blood transfusions after implementation. This is an improvement of 0.8%. The non-isolation haematology ward and the haematology and digestive oncology day hospital registered both +0.5% blood administrations correctly after implementation. Although this looks as a small improvement, it means a big gain in time and energy for the haemovigilance team who performs all corrective actions, it results in a better invoice of blood components and of course more patient safety when blood administration is scanned instead of only visually controlled by nurses or doctors.
Summary/Conclusions: The next step will be to develop these templates for paediatric patients. Paediatric blood transfusions are often prescribed in millilitres instead of units and, as such, some imperative changes need to be implemented in the templates for electronic prescription and administration of blood components. At request of nurses, ICT additionally developed an automatic link to register the patient follow-up during blood transfusion.

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COMPREHENSIVE ANALYSIS OF LIBERAL AND RESTRICTIVE TRANSFUSION STRATEGIES IN PICU

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Background: We prospectively compared restrictive and liberal transfusion strategies for critically ill children regarding hemodynamic and laboratory parameters.
Aims: Use of a more restrictive blood management program will result in a reduction of the number of transfusions and total blood transfused and will lead to improved outcomes and reduced complications as a result of PRBC transfusions.
Methods: One hundred eighty critically ill children who requiring packed red blood cells (PRBC) were randomized into a two groups: the restrictive transfusion strategy group (transfusion trigger <7 g/dl Hb) and the liberal transfusion strategy group transfusion trigger <10 g/dl. Basal variables including venous/arterial hemoglobin, hematocrit, lactate levels, stroke volume (SV) and cardiac output (CO) were recorded at the beginning and end of the transfusion. Oxygen saturation (SPO2), noninvasive total hemoglobin (SPHb), noninvasive total oxygen content (SPOC), perfusion index (PI), heart rate (HR) and systolic and diastolic blood pressures were assessed via the Radical-7 pulse co-oximeter (Masimo, Irvine, CA, USA) with the Root monitor, initially at the end of the transfusion. Demographic data, PRISM, PELOD, reason for transfusion, lengths of mechanical ventilator and pediatric intensive care unit stay and survival were recorded from medical charts. One hundred eighty critically ill children who requiring packed red blood cells (PRBC) were randomized into a two groups: the restrictive transfusion strategy group (transfusion trigger <7 g/dl Hb) and the liberal.
Results: One hundred sixty children were eligible for final analysis. The baseline hemoglobin level for the PRBC transfusion was 7.38 ± 0.98 g/dl for both groups. When the pre-transfusion and post-transfusion data were compared in both groups, the mean Hb level increased from 8.07 ± 0.76 g/dl to 10.33 ± 0.9 g/dl in group 1 ($P < 0.001$) and from 6.52 ± 0.30 g/dl to 9.05 ± 0.61 g/dl in group 2($P < 0.001$). At the end of the PRBC transfusion, cardiac output decreased by 9.9% in group 1 and by 24% in group 2 ($P < 0.001$). PI increased 10% in group 1 and 45% in group 2 ($P < 0.001$). Lactate decreased 9.8% in group 1 and 31.68% in group 2 ($P < 0.001$). One hundred sixty children were eligible for final analysis. The baseline hemoglobin level for the PRBC transfusion was 7.38 ± 0.98 g/dl for both groups. When the pre-transfusion and post-transfusion data were compared in both groups, the mean Hb level increased from 8.07 ± 0.76 g/dl to 10.33 ± 0.9 g/dl in group 1 ($P < 0.001$) and from 6.52 ± 0.30 g/dl to 9.05 ± 0.61 g/dl in group 2($P < 0.001$).
Summary/Conclusions: Restrictive blood transfusion strategy is better than liberal transfusion strategy with regard to the hemodynamic and laboratory values during the early period. PI also Restrictive blood transfusion strategy is better than liberal transfusion strategy with regard to the hemodynamic and laboratory values during the early period.

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Abstract has been withdrawn.

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COMPARISON OF AMOTOSALEN/UVA LIGHT PATHOGEN-REDUCED PLATELETS IN 100% PLASMA VS AMOTOSALEN/UVA LIGHT PATHOGEN-REDUCED PLATELETS IN PAS: *IN VITRO* FUNCTIONAL AND SURVIVAL PARAMETERS

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Background: Pathogen inactivation (PI) technology for platelet concentrates (PCs) has been described to enhance blood safety by reducing the risk for transfusion-transmitted pathogens transfusion associated graft-vs host disease. Furthermore, PI technology allows the storage of PCs up to 7 days. The usage of platelet additive solution (PAS) was reported to reduce the risk for non-hemolytic transfusion reactions and enhance platelet quality during storage. However, there is not much known about the impact of the combination of PI and PAS against the combination of PI and 100% plasma on the biochemical platelet quality parameters.
Aims: Analysis of the impact of Amotosalen/UVA pathogen inactivation technology on the biochemical parameters and functional activity of PCs suspended in 100% plasma compared to PCs suspended in 70% PAS and 30% plasma.
Methods: Apheresis-derived PCs were either pooled in 100% plasma (PCs_p, n = 30) or in 30% plasma + 70% PAS (SSP^+, Macopharma) (PC_{PAS}), n = 30). Each PC pool was split to obtain identical therapeutic PCs that were either control (CPCs) or treated with Amotosalen/UVA Pathogen Inactivation (INTERCEPT Blood System, Cerus B.V.) (PRPCs). *In vitro* parameters were analyzed on day 0, 3, 5 and 7 of storage. Biochemical parameters (pH, Glucose, L-Lactic acid and Citric acid concentration) were determined in PC supernatants post centrifugation at 5,000 rpm for 30 min.
Results: The pH of PCs in PAS was generally more stable than the pH of PCs in 100% plasma, also in pathogen reduced units. All 4 different units were at day 7 between pH 6.6 and 7.4; within the guardbands of the EDQM Guidelines (pH 6.4–7.4). The Glucose consumption rate in PCs suspended in 100% plasma did not change significantly after pathogen inactivation at the end of the 7d storage period (in PC – 41.36 mmol (5.9 mmol/day) and 38.80 mmol (5.5 mmol/day) in PRPC). In PC_{PAS}, the glucose consumption dropped to 23.84 mmol, and increased in $PRPC_{PAS}$ to 33.3 mmol. The glucose consumption rate was significantly lower in $PRPC_{PAS}$ compared to PRPC in 100% plasma.
Total lactate accumulation in PCs at day 7 of storage was significantly higher in PC/PRPC in 100% plasma compared to PC/PRPC in PAS. While a total concentration of 16 mmol/l was
Summary/Conclusions: More stable biochemical quality parameters have been observed in pathogen-reduced PCs in 70% PAS and 30% plasma compared to pathogen-reduced PCs in 100% plasma.

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INTRODUCTION OF PLATELET CROSS-MATCHING IN INDIA: TRANSITION FROM RESEARCH TO CLINICAL APPLICATION

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Background: Platelet is an important factor for hemostasis. Thrombocytopenia or low platelet count that often causes internal and/or external hemorrhage necessitates platelet transfusion. On a routine basis, platelets are issued in a group compatible manner. However in emergency, platelets of any group may be issued. But often there is less than expected rise in platelet count in recipient, even after transfusing quality products. The suspicion of platelet refractoriness arises then. To overcome this problem HLA or platelet antigen matched platelets are transfused in higher centers. However in developing world or resource poor setting where such matching is impossible platelet cross-matching is a simple methodology that ensures optimized platelet survival and increment in patients.
Aims: To implement platelet crossmatching as a routine practice for clinical benefits in refractory patients.
Methods: This is a prospective study of 7 months comprising of 5 adult patients of aplastic anemia who have developed platelet refractoriness. All were recipients of multiple platelet transfusions since long duration. Clinical decision to transfuse crossmatch compatible platelets was taken and random donor platelets (RDP) were matched using the Capture-P Solid Phase System (Galileo, Immucor Inc, USA) following manufacturer's instruction. As per the validation protocol reactivity strength of ≥40 was considered reactive or incompatible. For all patients more than twice the number of platelet units requested was subjected to crossmatch and compatible units were transfused under observation and documented. For all episodes Platelet Corrected Count Increment (CCI) was calculated at 1 and 24 h of platelet transfusions.
Results: A total of 67 platelet units were crossmatched for 5 patients (4 males and 1 female) of which 27 units were found to be compatible. Each patient received 4–6 units of compatible RDP without any untoward events. The mean CCI after 1 and 24 h of transfusions was 13,100 platelets × m^2/μl and 11,362 platelets × m^2/μl respectively. These CCI values were significantly higher than values observed after transfusions of uncrossmatched platelets (3,423 platelets × m^2/μl and 2,562 platelets × m^2/μl respectively). The mean platelet count increment with compatible platelets was found to be 26,543/μl.
Summary/Conclusions: This study describes the first clinical application of crossmatched platelets for transfusion in a tertiary care hospital in India. Initial standardization and validation were performed in 25 samples of normal voluntary blood donors. A total of 27 units (40.3%) were found compatible which indicates requirement of more platelet units to be crossmatched to transfuse an optimum hemostatic dose to these patients. The reaction strength cut off which was initially at 38 was increased to 40 after the validation process. Like the current study previous authors also described clinical utility of platelet crossmatch in refractory patients. However cost is a hinderance for most patients in developing countries. However we conclude that countries lacking a donor registry or without facilities of HLA or platelet antigen matched platelets may implement platelet crossmatching facility to manage thrombocytopenia and bleeding due to platelet refractoriness.

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APPROPRIATENESS OF PLATELET USE IN MATER DEI HOSPITAL, MALTA

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Background: Platelet use is increasing, mostly in patients with haematological disorders. An audit exploring the appropriateness of platelet transfusion is the first of its kind in Mater Dei Hospital, the main university hospital in Malta.
Aims: To evaluate clinical practice of platelet transfusion using an audit tool based on the Guidelines for the use of platelets published by the British Committee for Standards of Haematology (BSH), the primary aim being improvement in practice. This was performed with a special focus on patients with haematological conditions.
Methods: Data was collected over 10 weeks from the 5th October to 14th December 2015 and recorded on audit forms specifically designed for Haematology, Cardiac, ITU and Miscellaneous specialties. Data was analysed using a spreadsheet software programme.
Results: A total of 332 platelet transfusion episodes (episodes = all platelet units transfused within 24 h) took place over the 10 week audit period. The total number of units transfused amounted to 403, with an average of 5.84 platelet units transfused daily. 83% of the episodes were administered to Haematology patients, 6% to ITU patients, 3% to Cardiac patients and 8% fell in the Miscellaneous category. In the majority of episodes (84%), one platelet unit was transfused. In 12% two units were transfused. 59% of episodes took place in male patients, and 41% of episodes were transfused to female patients. 10% of episodes were transfused to children up to 10 years of age. 42% of transfusion episodes were transfused to patients between 60 and 80 years of age.
Within the Haematology cohort, 83% of episodes were given to in-patients and 13% to day care patients. 87% of the total episodes were transfused for prophylactic reasons (77% routine; 9% to raise platelet count prior to procedure). These included 6 episodes where prophylaxis was given for bone marrow aspiration or biopsy. For the greater part of the episodes occurring for prophylactic reasons (87%), one unit was transfused. In 9% of episodes, two units were transfused. 87% of the routine prophylaxis episodes were deemed to be appropriate (transfusion trigger 10 or 20

depending on patient condition including APML, use of antibiotics, anti-fungal therapy or antibiotics). 4% of episodes were given because the patient was bleeding. In 8%, no reason was given for the transfusion, and no documentation was available for 2% of episodes.

Summary/Conclusions: Since this platelet audit is the first to be carried out locally, it will serve as a benchmark for future audits. Appropriateness of platelet transfusion by the haematology specialty, the main users, is reasonable. More rigorous observation of guidelines however, as in the lack of necessity for prophylactic cover in bone marrow aspiration and biopsy, will improve appropriateness of use. It is noteworthy that new BSH guidelines with modified indications have been issued since the data was collected. Ensuring clinician awareness in this regard is important. A re-audit to assess compliance should be performed after an established period.

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ADVANTAGES OF TYPE AND SCREEN POLICY: PERSPECTIVE FROM A DEVELOPING COUNTRY!

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Background: The authors' center recently changed their pre-transfusion testing protocol from 'conventional' type and crossmatch with AHG crossmatch (Policy A) to type and screen with immediate-spin crossmatch (Policy B). RBC units were issued after compatible IS crossmatch as and when required instead of AHG crossmatch.

Aims: Study was done to compare the effects of change of policy from A to B over 1 year period on C/T ratio, RBC issue TAT, outdating of RBC, man hours consumption and finances.

Methods: This was a comparative prospective study conducted by department of Transfusion Medicine of a tertiary hospital-based blood bank in northern India. The policy-B was implemented in the department from January 2014. Relevant retrospective data for comparison for the previous 1 year, when policy-A was practiced, was derived from hospital information system. Parameters of comparison were:

1. Cross-match to Transfusion Ratio (C/T): Number of RBC units cross-matched/ Number of RBC units transfused
2. Turnaround time (TAT)= (Time of Issue − Time of Requisition)

Steps in policy A

included blood group of patient and donor, AHG crossmatch, labeling and reservation of compatible RBC unit(s) and finally issue of the unit at the time of requirement. Steps in policy B included blood group of patient and donor, followed by immediate-spin crossmatch at the time of issue of RBC unit.

3. RBC outdating = Total number of outdated RBC units/calendar year

This was calculated as number of RBC units discarded due to outdating of their shelf life during each study period.

4. Man-hours Utilization:

a. Type and Crossmatch man-hours = (AHG crossmatch + issue RBC units + reserve and un-reserve units) minutes

b. Type and Screen man-hours = (IS crossmatch + issue RBC units) minutes

These time durations in minutes were recorded by conducting 'time-motion' studies over a period of 1 week three times over and mean was calculated. Total man-hours consumed were calculated by multiplying this mean time per process with total number of RBC units issued in each study period.

5. Financial calculation: Financial calculation was done for each study period as follows: Cost of consumables and reagents per crossmatch X total cross-matches

Data was analyzed using SPSS (version 24.0, IBM, Bengaluru, India). P-value less than 0.05 was considered statistically significant.

Results: 23,909 and 24,724 RBC units transfused to patients admitted in the hospital during respective 1-year period of practice for policy A and B. There was significant reduction in C/T ratio (1.94 vs 1.01; P = 0.0001) and RBC issue TAT (79 vs 65 min; P = 0.007) with policy B. Expiry due to outdating reduced (37 vs zero), and man-hours (16% reduction) and finances (33% reduction) were saved.

Summary/Conclusions: Use of type and screen policy provides multiple advantages to all the stakeholders; blood banker, clinician, patient, and the hospital management.

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GRANULOCYTE TRANSFUSION IN NEUTROPENIC PATIENTS AND PATIENTS WITH INHERITED GRANULOCYTE DISORDER

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Background: Infections cause major mortality among persons with prolonged neutropenia related to HSCT and chemotherapy and among patients with granulocyte disorder. The granulocyte transfusion therapy, a logical approach in treating patients with severe life-treating infections

Aims: We want to demonstrate our granulocyte transfusions experience in children who have neutropenia or defects of the immune system and severe infections

Methods: We analyzed the retrospective data for the period of 04/2012–02/2016. All donors underwent granulocyte mobilization with G-CSF (3–5 μg/kg s.c.) 12–16 h prior to donation; granulocytes were collected on COBE Spectra, CaridianBCT, Version 6.1. The analysis was performed through Microsoft Excel, XLSTAT using the nonparametric Mann-Whitney's and Spearman's tests

Results: Was performed 169 granulocytes collections in 153 volunteered donors (82 women, 71 men), ages 18–58 years. The mean number of total WBC in the product was 38.4×10^9/l ($13.4–77 \times 10^9$/l; female-34.8×10^9/l, male-42.7×10^9/l). Only three donors (1.96%) had apheresis-related adverse effects (1-citrate toxicity, 2-circulatory reaction). 244 granulocyte transfusions for the treatment of 35 cases of infectious complications in 32 patients were included. Nosological structure of patients was: aplastic anemia-9 pts, AML-8, ALL-8, severe congenital neutropenia-4, chronic granulomatous disease-2, HLH-1, LCH-1, congenital dyskeratosis-1, Burkitt lymphoma-1. 21 patients had sepsis (6 with septic shock); the etiology of sepsis was: 8-bacterial, 6-fungal, 1-mixed, 6-unknown. 10 patients had multiple infectious focuses on the skin and mucous; 2 patients had pneumonia; 2 patients had appendicitis. The mean number of transfusions in case of infection was 7 (1–60). Each patient received transfusions from mean 5 donors (1–32); the granulocyte transfusions starting date of infection manifestation was 8 days (2–30); the mean dose of WBC on the transfusion was 11.6×10^8/kg ($2–30.7 \times 10^8$/kg). Increment of WBC was able estimated after 144 transfusions. Increasing the number of WBC was recorded after 96 transfusions (66.7%); the mean increment was 1.12×10^9/l (0.01–11.38×10^9/l). Efficacy was determined by the number of patients who survived an episode of infection and amounted to 80.6%; in the cases of sepsis, the efficacy of transfusions was 76.2%. The frequency of post-transfusion reactions was 22%, of which FNHR 8.3%; pulmonary complications 11.1%; the anti-HLA antibodies formation 2.7%.

Summary/Conclusions: According to our data, the mean total WBC in the collected product from men and women were significantly different. Perhaps the reason for lower cell products from women is to treat smaller blood volume. A multicenter, randomized, controlled study RING data was published. Because of limitations in this study, these results cannot interpret as proof that granulocyte transfusions are ineffective. In our center, the granulocyte transfusion therapy is useful, but the need and feasibility must be assessed critically in each situation. The lack of increment WBC after transfusion is not equivalent to lack of efficacy. In our study, we found a statistically significant association between transfused dose and increment of WBC. We deliberately did not estimate our data on the treatment of patients with sepsis and other severe infections between patients receiving and not receiving granulocyte transfusions because the need for granulocytes transfusions indicates more severe patients, when they have infections in a neutropenia or dysfunction of the immune system, and refractory to antibiotics

P-645

RED CELL SPECIFICATIONS FOR PATIENTS WITH HEMOGLOBINOPATHIES: A SYSTEMATIC REVIEW AND GUIDELINE

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Background: Red blood cell (RBC) transfusions remain essential in the treatment of patients with sickle cell disease (SCD) and thalassemia. Alloimmunization, a well-documented complication of transfusion, increases the risk of delayed hemolytic transfusion reactions, complicates crossmatching and identifying compatible units, and delays provision of transfusions. Guidance is required to optimize the RBC product administered to these patients.
Aims: To develop evidence based recommendations for red blood cell selection for patients with hemoglobinopathies
Methods: An international, multidisciplinary team conducted a systematic review and developed, following the GRADE methodology, recommendations to assist treating physicians and transfusion specialists in their decision to select RBCs for these patients.
Results: Eighteen studies, 17 clinical studies and 1 cost-effectiveness study were included in the systematic review. The overall quality of the studies was very low. In total 3,783 patients, 1,891 with thalassemia and 1,892 with SCD were included.
Summary/Conclusions: We recommend that ABO Rh K matched RBCs are selected for individuals with SCD and thalassemia, even in the absence of alloantibodies, to reduce the risk of alloimmunization. In patients with SCD and thalassemia who have developed clinically significant alloantibodies, selection of RBCs antigen negative to the alloantibody is recommended. In these patients, selection of more extended phenotype matched RBCs will likely further reduce the risk of alloimmunization. However, given the limited availability of extended phenotype matched units, attention should be given to ensure that a delay in transfusion does not adversely affect patient care.

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Abstract has been withdrawn.

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EVALUATION OF BLOOD REQUISITION AND UTILIZATION PRACTICES AT A TERTIARY CARE HOSPITAL BLOOD BANK IN ISLAMABAD, PAKISTAN

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Background: The significance of appropriate completion of blood request forms is frequently underrated by the clinicians which results in wastage and increased risk of inappropriate therapy. Similarly the judicious use of blood components can be assessed by the crossmatch to transfusion ratio (C:T), transfusion probability (%T) and transfusion index (TI).
Aims: The current study assessed the standard of completion of blood requisition forms and blood components utilization at a tertiary care hospital blood bank.
Methods: This was a cross-sectional, prospective study conducted at the Department of Blood Transfusion Services, Shaheed Zulfiqar Ali Bhutto Medical University Hospital, Islamabad, from Jan – Apr 2016. 5,957 consecutive blood request forms that were submitted to the Blood Transfusion Services between the study period were compiled and reviewed. The data was entered and analyzed using SPSS (IBM SPSS Statistics for Windows, version 20.0. IBM Corp., Armonk, NY, USA). The data were entered anonymously and recognized by a unique research ID. The request form was evaluated for the fullness of the data requested therein: patient's name; patient's PCN; patient's ward and bed; referring doctor's signature; referring doctor's name; type of blood components required; number of blood components required; a clinical history (defined as a clinical history and/or differential diagnosis); previous history of transfusions; patient's blood group; referring doctor's name and signature. These data should be present on 100% of requests if completed correctly. The red cell concentrates utilization practices were assessed using crossmatch to transfusion ratio (C:T ratio); transfusion probability (%T); and transfusion index (TI).
Results: During the study period, a total of 5,957 blood requests including 1,544 from mother and child healthcare centre (MCHC) were received. 3,509 (58.9%) were females and 2,448 (41.1%) were males. 3,895 patients (65.4%) were actually transfused while 2,062 (34.6%) were not transfused (only crossmatched). Out of 1,544 blood requests from MCHC, only 144 (9.3%) were transfused. The overall C:T ratio, transfusion probability and transfusion index calculated were 1.52, 65.38% and 0.65 respectively while for MCHC these values were 10.7, 9.3% and 0.09 respectively. A total of 5,957 blood request forms were analysed, out of which only 12.7% were completed. Parameters of BRF remained incomplete were patient's name; 31.9%, patients PCN; 7.3%, patient's age; 14.6%, patient's sex; 19.9%, type of blood components required; 10.4%, number of blood components required; 19%, date and time when components are required; 43%, patient's blood group: 21.6%, referring doctor's signature; 10.2. Previous history of transfusion (96.6%) and referring doctor's name (83.6%) were the most incomplete parameters. No patient's diagnosis was provided on 39% of forms and when a diagnosis was present it was abbreviated. Incomplete ward and bed information was found on 60.8% of forms.
Summary/Conclusions: Incomplete blood transfusion request forms create difficulties for the blood bank staff in comprehending the requests which may compromise patient safety. Similarly the efficiency of MCHC blood transfusion services is far from optimum. The Hospital Transfusion Committee can play a key role in solving this problem and thus improving the standards of Patient Blood Management.

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Abstract has been withdrawn.

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TO ANALYSE THE COMPLETE BLOOD COUNT AND HIGH PERFORMANCE LIQUID CHROMATOGRAPHY OF A SICKLE CELL DISEASE PATIENT FOR ESTIMATION OF IRREVERSIBLY SICKLED RED BLOOD CELLS

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Background: The triggers of blood transfusion in a sickle cell disease patient are haemolytic anaemia, acute painful clinical episodes and stroke which are secondary to the vaso-occlusive episodes caused predominantly by the irreversible sickle cells (ISC) sticking to the endothelium. The adhesiveness of the ISC is influenced by the presence of non HbS haemoglobins like HbA and HbF and reduced RBC hydration depicted by the high Mean Corpuscular Haemoglobin Concentration (MCHC) in the Sickle Cell Disease (SCD) RBCs which causes RBC membrane damage and endothelial adhesiveness.
Aims: To examine the role of low haemoglobin along with HbA, HbF and HbS and MCHC of the SCD patients to discern the causes of ISC in these patients.
Methods: The patients with a low Haemoglobin (Hb) on complete blood counts (CBC) were subjected to the High Performance Liquid Chromatography (HPLC) and sub-classified into control with normal HPLC (n = 21), SCD patients with Hb >9 g/g/dl (n = 42) and SCD patients with Hb <9 g/dl (n = 13) respectively. These parameters were statistical evaluated using Chi Square testing, ANOVA one way; Paired T testing and Correlation Regression analysis to evaluate the variations within the various Hb subtypes and MCHC within the groups.
Results: The SCD groups showed Haemoglobin levels HbA, HbA2, HbF and HbS significantly different between the SCD with Hb < 9 as compared to the Hb > 9

respectively (Chi-square statistic 9.98; $P < 0.05$). There was a significant difference between the individual Hb percentage Area under the curve (AOC) HbA, HbF and HbS respectively between the groups {SCD (Hb > 9 g/dl and <9 g/dl respectively); (P value <0.05; one tailed *t* testing in each Hb subtype respectively)}. There also was a significant difference of HbF levels between the control group {(HPLC n = 10 and each SCD group respectively); ($P < 0.05$; $t = -2.9$; df 10.1)(*t* testing one tailed)}.
There was significance difference between the MCHC values of the control group anaemic patients and SCD group Hb <9 g/dl and SCD with Hb > 9 g/dl{ANOVA testing ($P < 0.05$; df 2; $F = 4.01$)} however SCD groups (Hb > 9, Hb < 9) did not show a significant difference in MCHC {Paired *t* testing;($P > 0.05$; *t* 1.26; df 10.85)}. The mean Hb values did-not show a significant correlation with the Hb constituents.
Summary/Conclusions: Irreversible Sickle Cells in the SCD patients show a drift in the Hemoglobin subtypes (HbA, HbF and HbF) which are responsible for the haemolysis and SCD mediated veno-occlusive complications. The cellular hydration in the deformed SCD red cells are manifested in the form of significantly altered MCHC in proportion to the degree of anaemia in such patients. Irreversible sickle cell quantification may guide the transfusion management of the SCD patients to avoid unnecessary blood transfusions and prevent transfusion related morbidities. Novel therapies for sickle cell disease such as HbF inducing agents (hydroxyurea) or selective blocker of Gardos channels on RBC (Senicapoc) targets the ISC proportions.

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RED CELL ALLOIMMUNIZATION IN TRANSFUSED PATIENTS

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Background: Alloimmunization consists of the induction of immunity in response to foreign antigen(s) encountered through exposure to cells or tissues from a genetically different member of the same species. It is one of the major complications of regular blood transfusions, particularly in patients who are chronically transfused. Red blood cell (RBC) antibodies may result in clinically significant hemolytic transfusion reactions, difficulty in cross-matching blood, decrease in RBC survival and an increased transfusion requirement.
Aims: Identifying transfused patients in the South-Backa population who developed clinically significant red blood cell alloantibodies.
Methods: We analyzed the records of "crossmatch" results and antibody screening, in Blood Transfusion Institute of Vojvodina during 2016.
Results: Antibodies were found among 101 patients: 77 patients with single antibodies and 24 patients developed multiple antibodies. Distribution of antibodies: (i) from Rh system 55 patients: (4 anti-C, 2 anti-D, 30 anti-E, 19 anti-c); (ii) other clinically significant antibodies: 12 anti-K, 3 anti-Fy^a, 5 anti-Jk^a, 2 anti-S); (iii) 5 with usually not significant antibodies (3 anti-M, 1 Lewis, 1 Lutheran); (iv) 19 had antibodies of unknown specificity. Antibodies detected in the majority of patients (66.3%) had a specificity of Rh and/or the Kell system.
Summary/Conclusions: Approaches for prevention or treatment of alloimmunization range from provision of RBCs matched for all the major antigens associated with clinically significant antibodies to blood matched only for antibodies that have already been made. Database with a sufficient number of typed blood donors can resolve this problem.

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PRELIMINARY ASSESSMENT OF CROSS-MATCH-TRANSFUSION RATIO (CTR) AT YANGON GENERAL HOSPITAL, MYANMAR

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Background: The quality status of transfusion service in Myanmar is only at infancy stage. Satisfactory recruitment of voluntary donors (98%) is achieved only in 2016 at National Blood Center, Yangon but the nationwide voluntary donation is 75%. Cost for all the consumables have been supported by Government since 2015. Due to easy availability of blood and blood components without free of charge, hospital usages become increasing from 3,500 units (in 2012) to around 90,000 units (2016) per month.
Increasing demands from hospital do really threaten quality status of blood and blood products of National Blood Center which distributes 11 government hospitals.
To control clinical usage, local clinical guidelines were introduced in 2012. In 2016 February, locally designed software was installed at hospital blood issue units of hospitals where NBC distributes its products. In future, National blood center can get feedback analysis of usage from hospitals either rational or not.
Aims: Preliminary assessment of Cross match-Transfusion Ratio (CTR) of each ward of Yangon General hospital, which uses more than 5,000 units per month, was done to detect their efficiency on clinical usage.
Methods: At issue department of Yangon general hospital, manual record of cross match and issue units from each ward (January and February, 2017) of was done. Total of 13 wards used (6,508) units of red cells during this 2 months period.
Results: During January and February, 2017, 13 wards from Yangon General hospital requested to do cross matching for (7,588) units of red cells and total of (6,508) units were really used. Average CTR is (1.16). On reviewing the CTR of individual ward, medical ward is (1.1), Surgical ward (1.2), Emergency (1.2), Ortho (1.7), ICU (1.1), Radiotherapy (1), Oncology (1), Neurosurgery (1.1), Cardiac surgery (1.4), PMF (1.2), GI (1.2), Burn (1.5), Hand surgery (16.5).
Summary/Conclusions: According to this analysis, apart from hand surgery unit (CTR – 16.5), CTR of other 12 wards are lower than 2. This finding indicates that increasing usage of blood and blood components are not due to inefficient management and may be due to increased patients' admission.

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EVALUATION OF CROSS MATCHES RATES, BLOOD UTILIZATION AT FOUR HOSPITALS, SHIRAZ, IRANS

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Background: Patient blood management (PBM) is a major challenge to improve in the care of patients who need transfusion.
Aims: So, evaluation of blood request and utilization data for every ward of hospitals is essential to prepare criteria for transfusion, educate health-care professionals for better blood management and utilization, prevent unnecessary transfusion, and decrease blood wastage
Methods: In this cross sectional study, the data of cross matching, blood transfusion request, blood transfusion utilization, and wastage in four big hospitals of Shiraz, Iran in 2015 was calculated. Data was analyzed by comparison of proportions using MedCalc software 7.
Results: In this study, 87,796 units of blood were requested in all of the hospital wards that 36,725 units were transfused, and about 58.2% of blood units were left unused. The C/T ratio was equal to 2.45 on 2015. The C/T ratio was the highest in the maxillofacial ward (9.8) and obstetrics and gynecology wards (8.2) and the lowest in ICU ward (1.1)
Summary/Conclusions: This study showed that high C/T ratio compared with MSBOS tables So, preparing an audit for blood ordering, educating health professionals must be designed to reduce unnecessary blood ordering and optimize the use of blood and blood products

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LOW VS HIGH HAEMOGLOBIN TRIGGER FOR PERIOPERATIVE SEPARATION OF RED BLOOD CELL TRANSFUSION IN VASCULAR SURGERY: A RANDOMISED, ANALYST BLINDED, FEASIBILITY TRIAL (THE TV TRIAL)

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Background: In the surgical patient with cardiac disease, the Danish National Board of Health recommends administration of red blood cells (RBC) when haemoglobin levels drop below 8 g/dl, while in the vascular surgical patient, local guidelines often recommends higher triggers for RBC transfusion. A decrease in haemoglobin levels may affect blood O_2 transport with a potential for development of tissue ischaemia.
Aims: To assess whether two different haemoglobin triggers for RBC transfusion will separate post-operative haemoglobin levels, affects the number of transfused RBC units and the intraoperative tissue oxygenation determined by near-infrared spectroscopy. We also assess the occurrence of severe adverse events and evaluate post-operative coagulation competence by rotational thromboelastometry (ROTEM).

Methods: In a single-center, open-label trial design, fifty-eight vascular surgical patients (>40 years of age) awaiting open surgical repair of the infrarenal aorta or infrainguinal arterial bypass surgery in general anaesthesia undergo a web-based randomisation to one of two groups: perioperative RBC transfusion when haemoglobin decreases to 8 g/dl or RBC transfusion triggered by a haemoglobin 9.7 g/dl. Administration of fluid follows an individualised goal-directed strategy by optimising cardiac stroke volume and near-infrared spectroscopy determines regional oxygenation with optodes placed on the forehead and on the upper arm. The trial is powered to show a difference between the two groups for postoperative haemoglobin of 1.0 mmol/l (primary outcome), for the number of RBC transfusions by two units, and for tissue oxygenation by 6%. On the first and second postoperative day, myocardial injury is suggested by an increase in troponin-I above 45 ng/l. Renal failure is indicated by a creatinine-increment >26.5 mmol/l (or 1.5 times the preoperative value) or a drop in urine production <0.5 ml/kg/h over 6 h. Patients are followed up thirty and ninety days after surgery.
Results: Ninety days follow up on the last patient was set for March 8th 2017. Results will be presented at the 27th Regional Congress of the ISBT if the abstract were to be accepted.
Summary/Conclusions: This trial is expected to determine whether a RBC transfusion trigger of haemoglobin 9.7 g/dl compared to haemoglobin <8 g/dl results in adequate separation of postoperative haemoglobin levels, transfusion of more RBC units, and maintains a higher tissue oxygenation. The results are expected to provide information on the feasibility and the design of a future multicenter trial for evaluation of postoperative patient centered outcomes of mortality and serious adverse events.
Trial registration: ClinicalTrials.gov: NCT02465125.

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EVALUATION THE ROLE OF MSBOS IN DECREASING THE AMOUNT OF BLOOD UTILIZATION IN ELECTIVE SURGERIES

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Background: The maximum surgical blood order schedule (MSBOS) is a tool for transfusion services elective to predict blood transfusion in elective surgeries.
Aims: The aim of study is evaluating the blood request and cross match index after introducing MSBOS.
Methods: - · The MSBOS was created.according to the historical experience, list of common elective surgeries, number of red cell units ordered, number of red cell units which transfused by procedure. Then, cross match ratio and proportion of blood request to blood, transfusion rate were compared before and after MSBOS designing in 8 hospitals, shiraz, Iran in 2015 and 2016 were compared.
Results: The cross match ratio was significantly decreased from 2.42 to 2.13. The proportion of blood transfusion to request was significantly increased from 0. 48% to 0.56%.
Summary/Conclusions: It seems educating physicians to order blood according to the MSBOS can significantly improve blood transfusion utilization.

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Abstract has been withdrawn.

Haemorrhage and massive transfusion

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THROMBELASTOMETRY IS NOT ALWAYS ORDERED AS INTENDED

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Background: Thrombelastometry (TEM) is a form of whole blood functional clotting test for the diagnosis of coagulopathy in massively bleeding patients. One year after implementing TEM (ROTEM *delta* blood haemostasis analyser) at a large tertiary regional hospital a study was performed to ensure that TEM was being requisitioned as intended.
Aims: The aims were to assess whether TEM was being used appropriately by analyzing: (i) if patients were bleeding at the time of TEM testing; (ii) whether the bleeding was coagulopathic in nature; (iii) if all patients with massive bleeding as assessed by transfusion history had a TEM performed at time of bleeding.
Methods: All TEM tests requested during the year following TEM implementation in January 2016 were analyzed. Data were extracted from ROTEM analysers and the blood bank database. To estimate the extent of bleeding at the time of TEM ordering, the quantity of products transfused to each patient during the 24 h prior to the first TEM was enumerated. Coagulopathic bleeding was determined by the TEM results. Patients having massive bleeding were defined by transfusion of ≥10 red blood cell units (RBC) within 24 h.
Results: A total of 1,170 TEM were performed on 853 patients. Of these patients, 699/853 (82%) had only one TEM performed, 140/853 (16%) had 2–4 TEM performed; 14 (1.6%) patients had 5 or more (range 5–28). Abnormal TEM results were found in 466/1,170 (40%) of the tests in 346/853 (41%) patients. The most frequent abnormal result was prolonged clot time/clot formation time as found in 312/346 (90%) patients, while hyper fibrinolysis was seen in 49/346 (14%) of patients with abnormal results. For 507/853 (59%) patients all TEM parameters fell within normal ranges. Of patients who had at least one TEM, 255/853 (30%) received a transfusion within the 24 h prior to the first TEM (61/853 [7.2%] received ≥10 RBCs). The 255 patients were given a median of 5 (range 1–87) blood components; a median of 4 (range 1–38) RBCs, 4 (range 1–40) plasma units, and 2 (range 1–9) platelet units. In the 24 h following the first TEM, a further 181/853 (21%) patients were transfused. In total 436/853 (51%) patients received transfusion within 24 h surrounding their first TEM with a median of 2 (range 1–46) RBCs, 2 (range 1–54) plasma units and 2 (range 1–15) platelets. In total 95 patients met the criteria for massive bleeding; 34/95 (36%) of massively transfused patients did not have a TEM performed.
Summary/Conclusions: Within the first year of TEM implementation 853 patients had TEM performed; for 41% of these patients, TEM revealed a coagulopathic component to the bleeding. Only 30% of the patients received transfusion within the 24 h prior to their first TEM, with further 21% being transfused in the following 24 h, hence a high fraction of TEM are administered to patients without obvious need of transfusion. Although indicated, in total 34/95 patients with massive bleeding did not have a TEM performed. Additional educational efforts will be implemented to increase the awareness of the TEM.

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MASSIVE TRANSFUSION: FEEDBACK ON PRACTICE THROUGH HOSPITAL DATA REPORTS PROVIDES OPPORTUNITIES FOR PRACTICE IMPROVEMENT AND BENCHMARKING

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Background: Life-threatening critical bleeding (CB) resulting in massive transfusion (MT) occurs in many different settings, from obstetric to gastrointestinal to surgical bleeding and trauma, and may be unexpected. Opportunities to benchmark outcomes and provide information on practice improvements are relatively limited. The Australian and New Zealand Massive Transfusion Registry (ANZ-MTR) was established in 2011 to collect comprehensive clinical and demographic data on MT episodes. The registry now has complete data for over 5,000 MT cases from 25 participating hospitals.
Aims: To develop site-specific MT hospital data reports (HDRs) from the ANZ-MTR that facilitate patient care and practice improvement, including transfusion laboratory support, clinical care and governance. Specifically, to provide risk-adjusted clinical outcomes and data on blood utilisation for benchmarking with peer hospitals throughout Australia and New Zealand, and information to allow hospitals to measure and monitor compliance with national health care standards.
Methods: A survey to obtain feedback from participating sites on content and presentation preferences for the HDRs was conducted in 2016. Available ANZ-MTR data were analysed and graphs/tables/reports were developed to present data for each site and peer, Australia, New Zealand and overall, using statistical analysis software (STATA).
Results: In response to survey results, a new HDR template was developed that presented the data in a mix of easy to read formats including funnel plots, bar charts, box-and-whisker plots and tables, suitable for a wide range of users including

nurses, scientists, clinicians and management. Twenty-nine charts/graphs, two site-specific tables and four bleeding cohort-specific tables were produced, reporting on:

- Patient characteristics (including age, sex, hospital length of stay, ICU admission),
- Bleeding contexts, including a detailed examination of trauma, cardio-thoracic surgery, gastrointestinal bleeding and obstetric cohorts,
- Mortality (unadjusted and adjusted),
- Transfused fresh blood components, including FFP:RBC and PLT:RBC transfusion ratios,
- Other blood product use in large volume MT cases,
- Laboratory data (e.g. time to first coagulation and haemoglobin tests),

To automate the production of individual HDRs, a simple software macro script was written that populates each HDR with site-specific charts/graphs. Each HDR was then customised with a manually produced executive summary table.

Summary/Conclusions: Detailed information on management of critical bleeding and massive transfusion can be documented in hospital data reports, including transfusion laboratory support, clinical care and governance. This information is used at hospital level to initiate discussion, practice review, and examination of compliance with national standards and patient blood management principles, and to highlight areas for further investigation. It is also available for review by governance and policy bodies at state and national level to support practice improvement activities and highlight priority areas for future research in critical bleeding and massive transfusion.

P-658

Abstract has been withdrawn.

P-659

EVALUATION OF TIME IN THERAPEUTIC RANGE IN ANTICOAGULATED PATIENTS WITH NON-VALVULAR ATRIAL FIBRILATION

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Background: Despite the implementation of new oral anticoagulants (dabigatran, rivaroxaban, apixaban), vitamin K antagonists (AVK), such as warfarin and acenocoumarol are still the most widely used oral anticoagulant drugs in the treatment of non-valvular atrial fibrillation (NVAF). TTR (*Time in Therapeutic Range*) is a measure of the quality of anticoagulant effect of these drugs, and it is considered that the lower values of TTR are associated with the adverse effects of therapy.

Aims: The aim of this study was to determine the quality of AVK therapy by determining the TTR, and also to identify the main factors affecting the quality of the therapy.

Methods: We conducted a retrospective study of patients with NVAF treated with AVK, who were followed at the Institute for Blood Transfusion of Niš during the period January-December 2016. The effect of AVK therapy was determined by prothrombin time (PT) expressed as INR (International Normalized Ratio, INR = 1) from capillary blood of patients, which was measured on Trombotrack Solo (*Axis Shield, Norway*) and Thrombostat (*Behnk Elektronik, Germany*). The target INR was between 2.0 and 3.0. On the basis of all INR results for each patient we determined TTR using Rosendaal method.

Results: The study included 4.225 patients with NVAF who have done 34.336 INR controls. The mean value of TTR was 64.15 ± 18.53%. 27% of patients had a high quality of AVK therapy (TTR > 80%), while 39.72% of patients had TTR < 60%. The mean percentage of time with high risk of bleeding (INR > 3.0) was 18.1%, while INR was below the target INR (INR < 2.0) in 21.05% of the follow-up period, with high thrombotic risk (INR < 1.50) in 6.15% of the time. The most significant independent factors affecting the quality of AVK therapy are obesity, female gender and use of amiodarone.

Summary/Conclusions: In order to improve the quality of management of AVK anticoagulation we need proper monitoring and education of patients, but also successful teamwork with clinicians.

P-660

HYPERFIBRINOLYSIS DETECTED BY ROTEM IN TRAUMA PATIENTS

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Background: Hyperfibrinolysis occurs in acute trauma and is reported to be associated to poor outcome. However, the diagnostics of hyperfibrinolysis and treatment strategies are not evident.

Aims: The aim of this study was to identify and characterize trauma patients with hyperfibrinolysis detected by rotational thromboelastometry (Rotem), at arrival to hospital.

Methods: Karolinska University Hospital has approximately 800 trauma alarms annually, of those approximately 15% receive blood transfusions and 2% receive massive blood transfusion (≥10 red blood cell units within 24 h). During the period 2015–2016, Rotem was analyzed at arrival, together with routine coagulation analyses in trauma patients with on-going or expected bleeding.

Results: Results 36 patients (27 men/9 women), age 44 [18–93 median, range] with hyperfibrinolysis detected by Rotem (Extem ML > 15%) were identified. 26 had blunt and 10 had penetrating injury with Injury Severity Score (ISS) 31 [1–75 median, range]. Fibrinogen concentration (Gauss) varied from not detectable to 5.3 g/l, and correlated to Fibtem maximum clot formation (MCF) (R 0.95), but not to Extem% ML. INR was increased in 14/36 and APTT in 26/36. 27/36 (75%) received blood transfusions, of which 17 (47%) received massive transfusion.

n (n survival) ISS 1–25 (24 h/30 d) ISS 26–40 (24 h/30 d) ISS > 40 (24 h/30 d) Total ML 89–100%* 6(6/3) 6(4/2) 12(4/2) 24(14/7) ML 16–60% 8(8/7) 2(2/1) 1(1/1) 11(11/9) Total 14(14/10) 8(6/3) 13(5/3) (*1 deceased patient not included ISS not known).

Summary/Conclusions: In this study on trauma patients with hyperfibrinolysis detected by Rotem at arrival to hospital, 25/36 (69%) survived 24 h and 16/36 (44%) survived 30 days. 12/13 with high ISS had severe hyperfibrinolysis and poor (33%) 24 h survival as well as poor (17%) 30 days survival. Patients with severe fibrinolysis and a low ISS had 100% 24 h survival but only 50% 30 days survival. Further studies with inclusion of a matched control group are needed to determine if hyperfibrinolysis diagnosed at arrival has predictive value for the outcome.

P-661

AN ALGORITHM TO IDENTITY CASES OF SEVERE HEMORRHAGE IN ROUTINELY COLLECTED HEALTHCARE DATA

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Background: Many patients with a hematological malignancy have an increased risk of hemorrhages. Research addressing the causes of these hemorrhages, especially those on major hemorrhages, are hampered by the difficulty to find sufficient and representative cases of major hemorrhage. Unfortunately, electronic health records generally do not codify hemorrhages.

Aims: The aim of this study was to develop an algorithm that can be used to find patients who suffered from major hemorrhages (WHO grade 3 or 4) within electronic health records.

Methods: An algorithm was developed using electronic health record data of a cohort of patients with acute leukemia, who received platelet transfusions between June 2011 and December 2015 at the Leiden University Medical Center in the Netherlands. Chart review was performed for a stratified, random sample of observation days. Discriminative performance of three indicators was assessed: CT-brain, drop in hemoglobin level and transfusion need within 24 h. The cut off values for hemoglobin drop and transfusion need with the best discriminating capacity and CT-brain were entered in the final algorithm. The C-statistic was calculated and calibration plots were made. The algorithm will be externally validated in two other academic hospitals.

Results: The derivation cohort consisted of 255 patients comprising 10,638 observation days and chart review was performed for 353 days. The incidence of major hemorrhage was 0.22 per 100 observation days. The final algorithm consisted of information on CT-brain (yes/no), a hemoglobin drop of ≥2.8 g/dl and the need of six or more transfusions (yes/no). The C-statistic of the algorithm was 0.93 (95% confidence interval (CI) 0.86–0.99). The incidence of bleedings with all grades of severity

was 8.4 per 100 days. The algorithm for bleedings of all grades had a c-statistic of 0.54 (CI 0.53–0.55). The results of the external validation are not available yet.
Summary/Conclusions: An algorithm using information on CT-brain, hemoglobin drop and transfusion can accurately identify cases of major hemorrhage within electronic health care data. External validation will be performed.

P-662
TRANSFUSION OF RED BLOOD CELLS UNITS IN EMERGENCIES IN THE BLOOD TRANSFUSION INSTITUTE VOJVODINA

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Background: In many situations, there is urgent need for blood before completion of compatibility testing (ABO-Rh, antibody screen, and crossmatch). Pre-transfusion testing, prior transfusion red blood cells (RBCs) to patient, included: 1) determining the recipient's ABO and RhD blood group; 2) final validation of compatibility between the recipient's plasma and the selected donor red cells performing serological crossmatch which include an antiglobulin phase. The procedure takes about 45 min to 1 h. In many situations *there* are *urgent needs* for *blood* that do *not* allow *time* for *complete testing. Depending* on the *urgency* of the *clinical situation* physician can order: a/ABO-Rh type-specific, partially crossmatched blood; b/ABO-Rh type-specific, uncrossmatched blood; c/type O RhD-negative, uncrossmatched blood.
Aims: To determine the urgency level of ordering blood for transfusion in The Blood Transfusion Institute Vojvodina in period 2010–2013.
Methods: A retrospective analysis of data from the protocols and databases is done. We compared the number of blood units applied after ABO-Rh type-specific, partially crossmatched blood and all transfused units. There was no transfusion of type O RhD-negative, uncrossmatched blood.
If the result of partially crossmatched blood (patient's plasma and donor RBCs were centrifuged, the test were read macroscopically for agglutination) was negative, blood was applied to the patient. Compatibility testing was completed as soon as possible (retrospectively) by the gel technique (Liss Coombs, Bio-Rad). If the result was positive, transfusion was immediately stopped.
Results: During the period 2010–2013 a total of 140.861 (100%) *RBC units* were full cross-matching. Out of which 2.692 (1.91%) were applied to patients after ABO-Rh type-specific, partially crossmatched. There were no posttransfusion hemolytic transfusion reactions in both groups.
Summary/Conclusions: Urgent transfusion of RBCs is necessary and lifesaving for patients with severe hemorrhage or with life-threatening anemia. ABO-Rh type-specific, partially crossmatching could detect only a few unexpected antibodies such as *antibodies* in the blood group systems MN, P and Lewis. *Most* of this antibody is *not* considered *clinically significant*. Transfusion of RBCs without crossmatch (which include an antiglobulin phase) is associated with a risk of non-ABO alloantibody-mediated hemolytic transfusion reactions and may be more hazardous for patient. For patient who have been exposed to RBC antigens, transfusion of the ABO-Rh type-specific partially crossmatched blood can be dangerous to health of the patient. This overall risk is low, posing an acceptable risk in the urgent transfusion setting. This information could be important to physicians in assessing the risks and benefits of emergency RBC transfusions.

P-663
BLOOD LOSS CLASSIFICATION FOR OBSTETRIC HEMORRHAGE

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Background: The frequency of blood transfusion in an obstetric pathology can reach 30%. The quality of transfusion therapy can affect the level of maternal mortality, can result in complications or preserve women's reproductive health. The complications associated with errors in transfusion practices account for 26% of all complications, defects in the infusion and transfusion therapy during the postoperative period represent the bulk of the complications. Transfusion therapy procedures in obstetric practice include clinical, hemodynamic and laboratory parameters with definitions of bleeding rates and volumes that implements the principle of evidence-based medicine.
Aims: The aim of this investigation was to study the state of hemodynamic, hemostasis as well as volume and rate characteristics of the transfusion therapy of pregnant women to develop recommendation for obstetric hemorrhage.
Methods: In total 73 pregnant women with obstetric hemorrhage who underwent transfusion therapy were included in this study. These cases were divided into three groups: a retrospective cohort with traditional treatment (n = 44), prospective cohort with innovate treatment (n = 15), and a new algorithm test cohort (n = 14). Clinic and maternity status were evaluated, blood loss assessment based on the gravimetric method was conducted, the index of shock as well as the dynamics of laboratory parameters were calculated.
Results: According to the American College of Surgeons advanced trauma life support (ATLS) there are four classes of hemorrhages depending on the percentage of circulating blood volume loss, which also determines the timing of blood volume replacement. During our study we used the bleeding rate to classify the extent of hemorrhages. The following correlation between blood loss and bleeding rate has been discovered.
Class I hemorrhage involves up to 15% of blood volume. The rate of bleeding in this case is 20 ml/min.
Class II hemorrhage involves 15–30% of total blood volume. The bleeding rate has been 20–50 ml/min.
Class III hemorrhage involves loss of 30–40% of circulating blood volume. The rate of bleeding in this case is 70–150 ml/min.
Class IV hemorrhage involves loss of >40% of circulating blood volume. Bleeding is stopped or the rate of bleeding is about 150 ml/min
The found correlation between the class of blood loss and bleeding rate allows to initiate blood volume replacement and prevent complications of patients with the fourth class of blood loss (19.8%, $P < 0.05$) relative to the control group.
It has been also found that the concentration of lactate anion, total protein and albumin, the base deficit as well as the prothrombin complex activity are reliably informative hemodynamic and hemostatic values for infusion therapy.
Summary/Conclusions: Using the developed criteria to classify hemorrhages in patients with obstetric hemorrhage was proved to be effective in stabilizing three patients' conditions and achieving stable basic physiological constants for 30 min without any additional infusion-transfusion therapy of blood components. Furthermore, these criteria can help preventing the development of significant changes in hemostasis such as hemorrhagic shock, DIC syndrome, multiple organ failure, clinical and biological death.

P-664
LACK OF ALLOIMMUNIZATION IN RH(D)-NEGATIVE RECIPIENTS AFTER REPEATED RH(D)-POSITIVE RED BLOOD CELL TRANSFUSIONS

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Background: Alloimmunization is more common in some clinical circumstances and patient populations than in others. The immunosuppressive regimens associated with solid organ transplantation and hematologic malignancies are believed to substantially reduce the incidence of alloimmunization. Here we report the three case of Rh(D)-negative patients subjected to repeated stimulation with D antigen because transfused with Rh(D)-positive red blood cells (RBCs) during bleeding emergency or inventory shortage of Rh(D)-negative RBCs.
Aims: Search for alloimmunization in three Rh(D)-negative patients subjected to repeated stimulation with D antigen.
Methods: In order to ensure uniformity of assessment and management of requests for RBCs when it's appropriate to transfuse Rh(D)-negative patients with Rh(D)-positive RBCs our Blood Service implemented a specific procedure which includes a negative Indirect Antiglobulin Test (IAT) (Ortho-Clinical Diagnostics) before the initial transfusion and subsequently when the patients require further RBCs transfusion. We evaluated IATs trend in three patients: (i) a male patient (69 years old) received transfusion upon liver transplantation (December 2008), during a second hospital admission between 2010 and 2011 and after a bleeding episode during a septic shock (December 2015); (ii) a male patient (54 years old) kidney transplanted, has been transfused during repeated dialysis procedures (2015–2016) and following a hemorrhage in 2016; (iii) a male patient (70 years old) with chronic lymphocytic leukemia and anaemia after proctorrhagia was transfused in January 2016 for about 2 months.

Results: Patient 1 has been transfused with 4 Rh(D)-positive RBCs during liver transplantation. There were no repetitions of IATs up to the following hospital admission 2 years later when he has been transfused only with Rh(D)-negative RBCs for several months and the 10 IATs performed always resulted negative. In December 2015 patient's IAT resulted negative again, therefore, following an hemorrhagic event, the patient received 5 Rh(D)-positive RBCs more. The 6 IATs performed in the 20 following days (every 3.3 days in means) were always negative. Patient 2 has been transfused between February 2015 and December 2016 with 235 Rh(D)-negative and 67 Rh(D)-positive RBCs. After the first transfusion with Rh (D)-positive RBCs a total 27 IATs were performed in 3 months (every 2.85 days in mean), all the tests were negative. The patient 3 has received 34 Rh(D)-negative and 19 Rh(D)-positive RBCs between January 2016 and March 2016. Within 2 months following the first Rh(D)-positive RBCs transfusion 9 IATs were performed (every 6.3 days in means) and they were all negative.
Summary/Conclusions: For patient 1, in the months following the first stimulation with D antigen, no IATs were performed, therefore due to lack of data it is not possible to determine whether there was an onset of primary antibody response. However, the lack of secondary antibody response after subsequent antigen stimulation, suggests that there has been no immunization. For patients 2 and 3 the IATs remained negative despite the repeated stimulations in the following months. The lack of alloimmunization in these patients can be considered comforting in cases where it is necessary to transfuse Rh(D)-positive RBCs to Rh(D)-negative patients also repeatedly.

P-665

MASSIVE TRANSFUSION PROTOCOL – A RETROSPECTIVE ANALYSIS OF TRAUMA CASES IN A TERTIARY CARE HOSPITAL

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Background: Massive transfusion is defined as the replacement of one blood volume within a 24-h period. For practical purposes for adults of average size, this is roughly equivalent to 10 units of RBCs with any accompanying crystalloid, colloid, platelet or plasma infusions. An infusion of greater than four units of RBCs in an hour and ongoing use anticipated could also be regarded as a massive transfusion. The strategy for treating massive hemorrhages has changed in recent years. "Damage control" resuscitation methods have been developed to directly address the coagulopathy of trauma and prevent lethal triad of hypothermia, acidosis and coagulopathy. Most medical centers with high-level trauma services have adopted a massive transfusion protocol (MTP).
Aims: The main aim of this case study is to highlight the importance of MTP to provide blood products to hemodynamically unstable trauma patients in an immediate and sustained manner.
Methods: The study was conducted at the Department of Transfusion Medicine where we retrospectively analyzed the results of MTP activation cases over a period of 1 year from February 2016 to January 2017. Records were used to identify patients for whom MTP protocol was activated.
Results: We found that MTP was activated 8 times for 8 trauma cases and was successfully deactivated later. A total of 231 units of blood components were transfused with a mean of 28 blood components for each patient and a ratio of 1:1:1 for red cell, fresh frozen plasma and platelets, respectively. Our analysis is based on the actual transfusion of blood products and MTP of our hospital includes 4 units of RBC, 4 units of FFP and 4 units of platelets
Summary/Conclusions: There is paucity of data available to help organize and develop a data driven institutional MTP. However most studies and recent advances in trauma research recommend that patients with massive blood loss receive at least one unit of plasma & platelets for every red blood cell transfusion or simply 1:1:1.
A damage control protocol should be prepared by a to manage patients who have significant acute blood loss after trauma or during surgery. A multidisciplinary team, including specialists from the emergency medicine, trauma, critical care, transfusion medicine, nursing, pathology and anesthesia departments should be involved in creation, activation and implementation of such a MTP. The rapid infusion of the correct ratio of products not only reduces the chances of developing (or decrease the severity of) trauma-associated coagulopathy but also has been shown to improve survival and decrease overall usage of blood.

P-666

THE NECESSITY IN FRESH-FROZEN PLASMA THERAPY IN CASE OF WARFARIN OVERDOSAGE

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Background: Despite the appearance of new oral anticoagulants, warfarin is still the most commonly prescribed for the treatment and prophylaxis of thromboembolic complications. Though the medicine is widely used, the selection of clinically effective and safe dose is still a complicated task for the doctors of all specialties. Patients have a high risk of hemorrhagic complications. That is connected with the narrow therapeutic window. According to literature data, the incidence of hemorrhage associated with warfarin, is from 7.6% to 16.5% per year, including life-threatening hemorrhage, which occurs at a frequency of 1.3–2.7%.
Aims: The aim of the study is to evaluate the necessity of substitution blood transfusion therapy in case of hemorrhagic complications associated with warfarin
Methods: A retrospective analysis of the substitution therapy transfusion of fresh frozen plasma in patients of City General Hospital during the year was made.
Results: There were 181 patients who had blood transfusion therapy with the fresh-frozen plasma. 35 patients (17 men and 18 women), which makes 20.4%, had a hemorrhagic complication as an indication for fresh-frozen plasma transfusion. The gastrointestinal hemorrhage was the most common complication – 15 patients had it. 6 patients had macrohematuria, 4 had the hemorrhage from the injection site, 2 patients had the hemorrhagic stroke, 2 had hypodermic haematoma, subdural hematoma formed in 1 patient. There also was one case of uterine hemorrhage and a case of bleeding from hemorrhoidal tumor.
The average age of patients is 69.7 $\pm$ 11.6 (from 43 to 93 years old). The results of INR level examination are the following: 7 patients had no coagulation in analysis, the average level of INR in the rest of patients was 9.8 $\pm$ 4.78. 4.5 was the minimal INR index, when the fresh-frozen plasma transfusion was made. Three patients with the INR level >10 had no hemorrhagic manifestations at the time of transfusion.
The volume of fresh-frozen plasma transfusion was 4.64 $\pm$ 1.98 units in average. 19 patients with hemmorhagic complications, associated with warfarin therapy, needed an erythrocytic medium transfusion because of heavy blood loss. The average number of transfused doses was 2.73 $\pm$ 1.4.
Summary/Conclusions: The necessity of blood transfusion therapy of hemorrhagic complications caused by warfarin among the indications to fresh-frozen plasma therapy is 20.4%. Both men and women needed transfusion equally.

P-667

Abstract has been withdrawn.

Adverse events, incl. TRALI

P-668

CHALLENGES IN THE EVALUATION OF RECIPIENT PULMONARY EVENTS–THE EXPERIENCE OF A LARGE COLLECTION ORGANIZATION

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Background: Pulmonary transfusion reactions (PTRs) have gained increasing importance as serious transfusion-related adverse reactions (TARs). Despite definitions set by hemovigilance systems, the distinction between transfusion associated circulatory overload (TACO) and transfusion related acute lung injury (TRALI) poses diagnostic challenges. Further, many of the diagnostic tests in support of TACO are invasive, and some diagnostic information will be of little or no value if not performed shortly after the reaction. Blood Collection Establishments (BCE) must be notified of reactions thought to be due to attributes of the donor and be provided with sufficient information to classify the reaction, enable timely management of co-components of implicated units, and determine eligibility of implicated donors.

Aims: To identify obstacles to timely evaluation of PTRs.
Methods: Transfusion events reported in 2016 to a large BCE serving >500 hospitals and distributing 1.8 million blood components were reviewed. TARs are reported via a 4-page, electronic-fillable form. The form and ancillary documents (copies of reaction reports, imaging reports, hospital and BCE physician notes) are maintained in a file. Data collected include: Patient's age, gender, clinical history, admitting diagnosis, indication for transfusion, transfusion history; Reaction information: units involved, vital signs, signs and symptoms, risk factors for TACO/ALI, fluid balance, clinical course; Labs (O2 sat, BNP pre and post, CVP, Ejection fraction, WBC) and imaging studies.
Results: During 2016, 70 TARs were reported from 37 hospitals. 18 (25%) of the 70 TARs were reported by 2 large metropolitan teaching hospitals. PTRs accounted for 37 (52.8%) of reported events, with 34 suspected TRALI and 3 suspected TACO. After BCE physician investigation of 34 suspected TRALI cases, final classifications included: 12 TRALI (possible/probable), 8 TACO, 5 Possible TRALI/Possible TACO, 4 due to underlying condition, 4 TRALI unlikely, and 4 pending test results. All 3 reported suspected TACO reactions were classified as TACO. The mean period between reporting and final classification was 21.2 days (1–142 days). Review of 37 charts demonstrated the most frequent barriers to prompt PTR classification included incomplete (not reported or not performed) imaging reports (n = 16, 47%), clinical history (n = 14, 41%), reaction data (n = 13, 38%), lab data (n = 9, 25%), and treatment of reaction with clinical response (n = 7, 21%). Completeness of reporting was most often associated with hospitals staffed with transfusion medicine fellows or specialists, or transfusion safety officers.
Summary/Conclusions: The primary obstacle to the timely evaluation of PTRs is incomplete clinical, lab and radiographic information. Almost half of PTRs had missing or incomplete chest imaging reports. Other missing information ranged from 21% to 40% highlighting the challenges faced when classifying TARs. Hospital clinical staff reporting reactions may not understand the rationale for the requested information, and providing requested information is undoubtedly time consuming, factors likely contributing to incomplete reporting. However, procuring complete information is essential to accurate case evaluation, final classification, and appropriate donor management. Modification of the reaction report form for simplicity and ease of completion is currently under evaluation to facilitate obtaining more complete information from hospitals.

P-669

CASE REPORT: A STRONGLY REACTIVE ANTI-HNA-2 IN A HEMATOLOGICAL PATIENT RESULTING IN TRALI REACTION AND REQUIREMENT FOR HNA-2 MATCHED RBC AND PLATELET TRANSFUSIONS

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Background: Antibody mediated Transfusion Related Acute Lung Injury (TRALI) is a transfusion reaction associated with high morbidity, and fatalities are reported in 10% of cases. TRALI is typically related to the presence donor related anti-human leukocyte antigens (HLA) or anti-human neutrophil antigens (HNA) antibodies in the blood products. These antibodies are usually a result of maternal immunization and the use of male only plasma have dramatically reduced the incidence of TRALI. A small proportion of TRALI cases is associated with the presence of granulocyte reactive antibodies in patient serum interacting with transfused residual leukocytes present in the blood products (reverse-TRALI). HNA-2 (CD177) is a neutrophil specific antigen located in the neutrophil cell membrane. Approximately 5% of the population lack the expression of HNA-2 on their neutrophils. The genetic nature HNA-2 deficiency is not yet established and HNA-2 deficiency has not been associated with any medical condition. Anti-HNA-2 antibodies are however known to cause severe TRALI reactions.
Aims: Leucocyte reduction was thought to eliminate cases of reverse-TRALI however; we here describe a case of a strongly reactive anti-HNA-2 in a patient resulting in a reverse-TRALI after transfusion of leukoreduced blood products leading to a requirement for HNA-2 matched blood products.
Methods: A multiparous female patient was admitted to the department of hematology. A transfusion dependent chronic hematological disorder was diagnosed and during transfusion of the first unit of leukoreduced red cells, she developed a severe clinical TRALI reaction. Samples from the patient and the suspected donor were referred to our laboratory. Sera were tested with the granulocyte agglutination test (GAT), granulocyte immunofluorescence test (FLOW-GIFT), Luminex based LabScreen Multi, and the Monoclonal Antibody Immobilization of Granulocyte Antigens assay (MAIGA). Screening for anti-HLA antibodies were performed using both Luminex and ELISA based methods. A flowcytometric method for HNA-2 typing using a monoclonal anti-CD177 antibody was established for donor screening.
Results: Donor serum was negative for all tested specificities. Granulocyte cross-match confirmed incompatibility between patient serum and donor granulocytes. The presence of a strongly reactive anti-HNA-2 in patient serum was detected and suggested an antibody mediated reverse-TRALI. All samples were anti-HLA antibody negative. HNA-2 screening of 500 donors was established. Nineteen donors were HNA-2 negative (3.8%) and HNA-2 matched blood products were subsequently transfused with no sign of transfusing reactions.
Summary/Conclusions: We found a patient specific anti-HNA-2 antibody resulting in a severe case of reverse-TRALI and the need for HNA-2 matched blood products. The case illustrates that reverse-TRALI, albeit rare, still can be seen even in the era of leukoreduced blood products

P-670

THE CHANGING LANDSCAPE OF LABORATORY INVESTIGATION OF TRALI – AN AUSTRALIAN EXPERIENCE

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Background: The landscape of laboratory investigations for TRALI, one of the leading causes of transfusion morbidity & mortality, has changed. No longer are HNA antibodies in donor plasma a common attributable cause, particularly following the introduction of risk reduction measures. Since 2007, The Australian Red Cross Blood Service (Blood Service) has implemented internationally recognised TRALI risk reduction strategies by introduction of predominately male clinical FFP with the target of 100% of clinical plasma from male donors (achieved 2013) and 100% of plateletpheresis donations from male donors (achieved 2016).
From 2000 to 2016, the Blood Service in Queensland, Australia investigated 80 potential TRALIs. In a previous retrospective analysis, 39 of these were determined to be TRALI, 17 possible TRALI and 24 cases were excluded (12 not TRALI, 12 insufficient information). This is an average of 4.7 TRALI reports/year (range 2–8), a number that has not changed dramatically since the introduction of various risk reduction measures.
Aims: To discuss the changing landscape of laboratory investigation of TRALI within a reference service in Australia, and explore possible areas for further research and development to allow more understanding of the pathogenesis of TRALI.
Methods: TRALI/ Possible TRALI cases referred to the Blood Service in QLD from 2000 to 2016 were reviewed with particular focus on the outcomes of laboratory investigation as opposed to clinical or product factors. Data was collected for cases where causes other than donor HNA antibodies are suggested but unconfirmed.
Results: Of the cases included in the TRALI and Possible TRALI categories (n = 56), 15 cases had neutrophil reactive antibodies in one or more donors (9 with HNA specificities). Six patients had neutrophil reactive antibodies, with donor specific HNA antibodies detected in one case. HLA antibodies (class I/II) were common in both patients and donors. Nine (16%) cases demonstrated no laboratory evidence to support the clinical diagnosis of TRALI.
Prior to 2007, TRALI investigations commonly identified neutrophil reactive antibodies in implicated donors (57% of cases had a neutrophil reactive antibody (n = 15, 6 with specificities of HNA-1a & HNA-3a). Following risk reduction measures, finding a HNA antibody in donor product is less common (13%, n = 4 with specificities of both IgG & IgM classes HNA-1a, HNA-2 and HNA -5b). Meanwhile, the number of reported TRALI cases per year holds steady and the focus of investigations into further risk reduction moves to other biological factors as potential causes.
A number of cases have been marked as "unresolved/interesting" by our laboratory. These cases include patients with HNA specific antibodies, IgM HNA antibodies in patients & donors, unidentifiable BRMs/ immune complexes causing demonstrable cell damage & HLA antibodies in patient only.
Summary/Conclusions: Laboratory investigations are integral to the elucidation of TRALI causes and to donor management, however there are many factors at play and not all are well understood or investigated even in the reference setting. These cases illustrate the need for further research into immune causes of TRALI; including the role that complement, other immune complexes and BRMs may play now that there is a lower prevalence of donor HNA antibodies detected.

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TRANSFUSION RELATED ACUTE LUNG INJURY (TRALI) – A CASE REPORT

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Background: TRALI is a rare but potentially fatal complication of blood product transfusion. It is an acute immune mediated transfusion reaction resulting in non-cardiogenic pulmonary edema during or within 6 h of transfusing plasma containing blood products. TRALI is a clinical diagnosis made by the exclusion of other causes of acute respiratory failure. Only a few cases have been reported in Sri Lanka.
Aims: TRALI presents a challenge in diagnosis as well as management. We report the case of a 40-year-old female who presented with features of TRALI after FFP transfusion and the consequent management of this patient and the implicated donor.
Methods: A forty year old pregnant woman admitted to the hospital at term for delivery. She was a primigravida with gestational diabetes mellitus on dietary control and severe scoliosis with bilateral lower limb weakness. She was on Enoxaparin for DVT prophylaxis for 2 months. Before the expected date of delivery the patient developed dribbling and an emergency Caesarean section was decided. She was transfused with five units of FFP over 2 h. One hour after the completion of transfusion patient developed fever (99.4 F), decreased SPO2, stridor and dyspnea. She was intubated and sent for the emergency LSCS under general anesthesia. Following surgery she was extubated and sent back to the ward with oxygen support. In the ward saturation started declining and the patient was transferred to the medical intensive care unit (MICU). With this clinical picture, heart failure, lower respiratory tract infection or transfusion reaction were considered as differential diagnosis. ABO incompatibility was excluded at the blood bank. In the MICU, patient's pulse rate and blood pressure was stable and she was afebrile. She was treated with steroids and diuretics. Chest X- RAY revealed bilateral pulmonary infiltrates, more on the left side. 2D ECHO and ECG were normal. IV antibiotics were started suspecting bronchopneumonia. No signs of fluid overload was detected. On the following day the chest X-RAY was repeated and it was normal with clear lung fields. Patient's shortness of breath also improved and oxygen support was removed. IV antibiotics were omitted. Patient recovered completely on the 4th day and was subsequently discharged.
Results: The FFP donors were traced. Among them two were multiparous female donors. Serum of the two female donors were tested for HLA antibodies using Lymphocytotoxicity method. In one donor HLA class 1 IgM antibodies were detected with PRA positivity. Specificity not identified and leucocyte crossmatch was compatible. The particular donor was removed from the donor pool.
Summary/Conclusions: TRALI is an acute life-threatening transfusion reaction often under diagnosed, underreported and inappropriately managed. It is of utmost importance to improve awareness about TRALI and appropriate use of blood components. Decisions of the transfusion services to use male only plasma and prepare apheresis platelets from males and nulliparous females will further support to minimize TRALI cases.

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TRANSFUSION-RELATED ACUTE LUNG INJURY IN A WOMAN WITH DIABETES MELLITUS DURING APHERESIS PLATELET TRANSFUSION – CASE REPORT AND LITERATURE REVIEW

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Background: Transfusion-related acute lung injury (TRALI) syndrome is a rare disease and difficult to be found with a 1:1,200–1:190,000 incidence. Clinically it is often misdiagnosed and underreported. TRALI is defined as new acute lung injury (ALI) that occurs during or within 6 h of transfusion, not explained by another ALI risk factor. The symptoms include hypoxemia ($PaO_2/FiO_2 < 300$ mmHg, $SpO_2 < 90\%$ on room air), appearance of bilateral lung infiltrates on a chest X-ray and no evidence of left atrial hypertension. The mechanism may include factors in unit(s) of blood, such as HLA antibody, HPA antibody and biologic response modifiers.
Aims: This study aims to elucidate the role of TRAIL in the process of apheresis platelets transfusion.
Methods: We observed the reports from CBC and pulse oximetry based on Canadian Consensus Conference TRALI (TABLE 1) and the bilateral lung infiltrates on a chest X-ray (Figure 1).
Results: Here we described a case of TRALI syndrome in a 69-year-old woman with diabetes mellitus. Her laboratory data revealed pancytopenia. CBC report as follows is WBC:1.27 $\times$ 10^3/μl, Hb:9.9 g/dl, Platelet: 23 $\times$ 10^3/μl. Because of thrombocytopenia, we performed 2 unit apheresis platelet (equivalent to 24 units platelet concentrates) transfusion. Severe hypoxia due to pulmonary edema developed half an hour after starting the apheresis platelet transfusion. The data shows SpO_2:73.1% and PaO_2/FiO_2:80.9 mmHg. Doctors suspected patients with TRALI that the implementation of intubation therapy, and informed the blood bank. We reported the data to Kaohsiung blood center, and the patient corresponded with the Canadian Consensus Conference TRALI. In order to ensure the safety of transfusion, we performed human leukocyte antigen (HLA) typing and set up the HLA-A and HLA-B data to find the similar type from the donor. Because of emergency situations of the patient, we performed leukocyte-reduced apheresis platelets transfusion first. Clinical anti-HPA antibodies detected in blood component allowed to identify the immune-mediated TRALI.
Summary/Conclusions: Clinical occurrence of TRALI should immediately stop the blood transfusion and give symptomatic therapy. TRALI is a life-threatening complication of transfusion. Each suspected case of this syndrome should be reported to blood center in order to obtain the most perfect blood transfusion.

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MODELING THE EFFECT OF PLATELET CONCENTRATE SUPERNATANTS ON ENDOTHELIAL CELLS: FOCUS ON ENDOCAN/ESM-1

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Background: Platelets are prone to activation and the release of Biological Response Modifiers (BRMs) under storage conditions. The transfusion inflammatory reaction in the vascular compartment involves endothelial cell activation due to cell-cell interactions and BRMs infused with the blood products. ESM-1/Endocan is a proteoglycan secreted by endothelial cells under the control of proinflammatory cytokines.
Aims: We aimed to measure Endocan activity in supernatants of platelet components (PCs), implicated in Serious Adverse Reactions (SARs) or not (no.AR), sampled at different stages during storage.
Methods: Platelet function was assessed by quantification of soluble CD62P and endocan. Functional testing of PC supernatants was performed on EA.hy926 endothelial cells *in vitro* by exposing them to PC supernatants from each group (no.AR or SARs); EA.hy926 activation was evaluated by their production of IL-6 and Endocan.
Results: Platelet endocan secretion was not induced in response to platelet surface molecule agonists, and no significant correlation was observed between sCD62P and endocan concentration after platelet activation. However, we observed a significant increase in the secretion of IL-6 and endocan following EA.hy926 activation by all PC supernatants. IL-6 and endocan secretion were significantly higher for cells stimulated with SAR than those stimulated with no.AR PC supernatants, regardless of the time in storage.
Summary/Conclusions: The correlation between the secretion of endocan and that of IL-6 by endothelial cells suggests that endocan can be used as a predictive marker of inflammation for the quality assessment of transfusion grade platelets.

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SOLUBLE CD40-LIGAND LEVELS IN PLATELET COMPONENTS IS ASSOCIATED WITH TRANSFUSION ADVERSE REACTIONS IN A SUBGROUP OF RECIPIENTS IN A MIXED THRESHOLD AND HIT MODEL

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Background: Platelet-derived soluble CD40-Ligand (sCD40L) is often associated with serious adverse reactions (SARs) following platelet component (PC) transfusion.

Aims: However, it is not clear whether high levels of sCD40L is always associated with SARs in recipients.
Methods: We thus collected samples from 9,206 consecutive PCs after processing and the 2,850 for which sampling was possible on the day of delivery to the patient and assessed sCD40L levels in all. Proactive hemovigilance identified 140 SARs. A mathematical model identified a threshold above which there was a significant association between sCD40L levels and SAR and allowed the elimination of entropy within the large dataset.
Results: Values for 40% of single donor apheresis (SDA)-PCs and 18% of whole blood, buffy-coat-derived, pooled PCs (PPCs) that resulted in SARs were equal to or below the threshold, whereas values for 18% of SDA-PCS and 46% of PPCs that did not lead to a reported SAR were equal to or above this threshold, indicating that sCD40L is not always responsible for pathogenicity in patients.
Summary/Conclusions: The thresholds were more discriminant for PPCs than SDA-PCs.

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TRANSFUSION ADVERSE REACTIONS IN PATIENTS TREATED BY PLASMA EXCHANGE

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Background: Patients with thrombotic microangiopathies (TMA) suffer from thrombosis in their capillaries and arterioles, which is often triggered by endothelial injury. Plasma exchange (PEX) represents the major treatment of patients with TMA. However because the administration of PEX is frequently required, transfusion adverse reactions (AR) are of main concern.
Aims: Our aim was to evaluate the registered ARs that occurred in patients with or without TMA treated with PEX during a period of 6 years in hospitals in the Auvergne Rhône Alpes area.
Methods: The study included patients with reported AR in hospitals in this area from January 1st 2010 to December 31st 2015. Each AR was registered in the national haemovigilance database system. The type of pathology, the severity of the AR, the clinical course and outcome, and the imputability of the blood product were studied.
Results: In total 6,612 AR were reported, of which 152 (2.3%) occurred upon the administration of fresh frozen plasma (FFP) and 74 (1.1%) of PEX. Of the 59 patients with PEX, 35 were females (59.3%) and 24 males (40.7%), with a mean age of 46 years. Among the 64 PEX reports in 49 recipients, 10 patients had TMA with thrombotic thrombocytopenic purpura, 18 with haemolytic uremic syndrome, one had an autoimmune disease and one patient was registered with a Haemolysis, Elevated Liver enzyme and Low Platelet count syndrome (HELLP). In 19 patients, the diagnosis of the TMA was not specified. Among the 74 AR reports, allergy was the most frequent AR (57 reports, 77.0%; including 50 reports in patients with TMA) followed by febrile non haemolytic transfusion reaction (6 reports) and the transfusion associated circulatory overload (3 reports). The 57 allergic ARs were reported in 43 recipients. Forty nine allergic ARs were non severe (86.0%) and 8 severe or life threatening (14.0%). No deaths were reported. The following cutaneous and/or mucous symptoms were predominant: urticaria (44 cases), rash (20 cases), pruritus (8 cases) and angioedema (5 cases). In allergic AR reports, respiratory symptoms were observed in 11 cases, gastrointestinal symptoms in 5 and shock in 7. Nine allergic ARs were certainly associated with FFP (15.8%). Imputability of FFP was probable or possible in 48 allergic ARs (84.2%). The recurrence of allergic AR was very frequent: 2 reports were registered in 5 patients, 3 in 2 patients and 6 in one. A prophylactic treatment or a treatment of the allergic reaction was rare in these reports.
Summary/Conclusions: Allergic ARs are frequently observed upon PEX which are predominantly performed in patients with TMA. The allergic AR can be severe and, in some patients, recurrences were observed. Because of the frequent PEX administration with numerous FFP units in patients with TMA, a very good survey of AR, particularly allergic reaction, is needed. A prophylactic treatment of allergic reactions may be beneficial to introduce in patients to avoid allergic AR.

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Abstract has been withdrawn.

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PAIN EPIDEMIOLOGY IN IMMEDIATE TRANSFUSION REACTIONS

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Background: Many symptoms are described in the clinical signs of immediate transfusion reactions. Some are clearly part of diagnoses clinical settings, but others are side effects which are not so well understood. Pain is both, sometimes awaited when there is haemolysis but also found in other reactions. Furthermore, some authors have described the acute pain transfusion-related (APTR) as a diagnosis *per se*, with pain as the key symptom.
Aims: Because of its age and its completeness, including the reporting of pain symptoms since its beginning, we decided to study the pain epidemiology in the whole French haemovigilance database.
Methods: ANSM, the French agency in charge of haemovigilance, produced a data retrieval of all the reported transfusion reactions occurring between 2000 and 2015 which include pain. This set of data could then be compared to the complete database. In order to be more pertinent, the study was focused only on immediate events with transfusion imputability at least possible.
Results: There were 122,776 reported reactions in the whole database during the study period, involving 6,563 with at least a pain symptom (5.3%). Keeping only the immediate and imputable events, pain is present in 4,806 of 61,185 (7.9%). Main pain localisations are lumbar (28%), abdominal (23%), head (15%), and thoracic (12%). While pain frequency is 6.8% in minor events, it rises to 12.2% in severe ones. Platelets transfusions are more incriminated with a pain frequency of 10.8% vs 7.1% for red cells and 3.2% for plasmas. A higher frequency of 12.0% is confirmed in case of haemolysis (29.1% in case of sickle cell disease), but the most surprising data is a frequency of 80.5% when the diagnosis is concluded "unknown".
Summary/Conclusions: This study provides useful data about pain in immediate transfusion reactions. But its main result is the very high frequency in unacknowledged events. A possible confusing effect of pain presence may be considered because it is mostly absent of classical clinical settings. However, such a high level can also be seen as an evidence of the APTR reality.

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Abstract has been withdrawn.

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ANAPHYLACTIC TRANSFUSION REACTION IN A HOME TRANSFUSION PATIENT

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Background: Transfusion remains an important supportive care for improvement of quality of life in patients with Myelodysplastic Syndrome (MDS). As transfusion is a relatively safe procedure, the option for home transfusion appeals to these patients. In the Northern Health and Social Care Trust, Hospital Diversion Nursing Team (HDNT) provides home transfusion services. HDNT nurses are appropriately trained for home transfusion.
Although transfusion is safe, anaphylactic transfusion reactions can rarely occur (incidence 1:20,000–50,000). As with any allergic transfusion reaction, anaphylaxis occurs most commonly during the transfusion of plasma or platelets.
Aims: Awareness of serious transfusion reactions.
Methods: Here we report a case of anaphylactic transfusion reaction in a home transfusion. An 80 year old lady diagnosed with MDS was transfused with a 7 day old pooled platelet unit at home under the care of HDNT. This patient required regular platelet transfusion due to recurrent WHO grade 2 and above bleeding. In addition, her baseline platelet count was less than 10×10^9/l.
Oral chlorphenamine 4 mg was given 20 min prior to transfusion. Vital signs were recorded prior to transfusion and 15 min from the time of administration. The observations were heart rate (HR) 79, 76/min, blood pressure 136/72, 130/60 mmHg, temperature 36.4, 36.2°C, respiratory rate 18, 18/min and oxygen saturation (SpO2) 95, 94% on air respectively.

After peri-transfusion vital observations, the patient complained of itch on her lower back. HDNT nurse examined the patient and noted rash which was spreading. Transfusion was stopped immediately and medical advice was sought. During this time, the nurse noticed that her breathing pattern had changed with decrease in conscious level associated with dusky lips and perioral swelling.
The nurse called the ambulance, administered adrenaline 0.5 mg (1:1,000) intramuscularly and commenced CPR. Following one cycle (30 compressions), deep breaths were noticed. At that time HR was 80/min and SpO_2 was 90%.
Ambulance arrived and paramedics subsequently administered chlorphenamine and hydrocortisone. On arrival to hospital, the patient was conscious, vital observations were stable except a mild tachycardia of 100/min. During her hospital stay, the rash and tachycardia resolved with regular chlorphenamine and hydrocortisone. This patient was discharged home 2 days later.
Results: The investigations for adverse transfusion reaction were performed. Clerical checks were correct. There was no evidence of haemolysis. DAT was negative on both pre and post-transfusion samples. The partially transfused platelet unit was sent for culture in Microbiology Department. There was no growth from the platelet pack. Historical IgA levels were normal. The final diagnosis of anaphylactic transfusion reaction was made. Following this episode, the patient continued to have platelet and red cell transfusions at hospital, initially with premedication. Later, the patient received washed single donor apheresis platelets and no further reactions reported since.
Summary/Conclusions: Although home transfusion is convenient for patients and provides better quality of life, serious transfusion reactions could develop unexpectedly. All home transfusion staff must be suitably trained and prepared for any serious reaction at home.

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INCIDENCE OF BLOOD TRANSFUSION REACTIONS IN A TERTIARY CARE HOSPITAL

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Background: As a result of effective hemovigilance and blood management programs that decreased the risk of noninfectious complications of transfusion the blood supply is safer today than at any time in history. Hemovigilance is the systemic surveillance of adverse reactions and adverse events related to transfusion with the aim of improving transfusion safety. One of the main purposes of developing a hemovigilance program is reporting of transfusion-related adverse events in order to identify transfusion hazards and demonstrate the effectiveness of interventions. It is widely believed that the major noninfectious complications of transfusion are both underrecognized and underreported.
Aims: The main aim of this study was to determine the frequency and type of blood transfusion reactions in our hospital patients as a part of the hemovigilance program.
Methods: We re-evaluated all reported blood transfusion reactions that were collected between March 2015 and November 2016 (21 months) at the Department of Transfusion Medicine in the Max Super Speciality Hospital in Vaishali, Ghaziabad. The physicians and nurses monitored the patients after the start of each transfusion for the occurrence of any blood transfusion reaction and reported the results of all transfusion to the department of transfusion medicine regardless of whether an adverse blood transfusion occurred via standard blood transfusion reaction form. All the blood transfusion reactions were evaluated in the blood bank and classified using standard definitions.
Results: Of a total of 13,921 units of transfused blood components 14(0.10%) transfusion reactions occurred. Out of 7,279, 3,709 and 2,933 red blood cell, fresh frozen plasma and platelet concentrate transfusions 5, 5 and 4 (0.069%, 0.135% and 0.136% incidence) adverse reactions were observed, respectively. Regarding the clinical characteristics of the adverse reactions after red blood cell transfusions febrile non-hemolytic transfusion reactions and allergic reactions occurred. All the fresh frozen plasma and platelet concentrate transfusion reactions were allergic reactions.
Summary/Conclusions: As with many necessary medical therapies, adverse effects cannot always be accurately predicted or avoided. It was found that the overall incidence of transfusion reactions in our hospital was less (0.1%:1 in 1,000) as compared to the data with other studies which shows an overall incidence of 0.25%(1 in 250). It is important that the transfusing physician is aware of risks when discussing the need for transfusion with a patient. Informed consent for transfusion should include a discussion of the risks of infectious disease and serious noninfectious complications. Furthermore, medical staff administering blood components should be well aware of the signs and symptoms of possible reactions. These staff should be prepared to mitigate the current episode and prevent future similar reactions when possible. Early recognition, prompt cessation of the transfusion, and further evaluation are key to a successful outcome.

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RETROSPECTIVE EVALUATION OF ADVERSE TRANSFUSION REACTIONS FOLLOWING BLOOD PRODUCT TRANSFUSION

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Background: The inherent risk of a transfusion reaction, ranging from mildly discomforting to life-threatening, is present with every unit of transfused blood product. Identification of the adverse reactions will help in taking appropriate steps to reduce their incidence and make blood transfusion process as safe as possible.
Aims: To determine the frequency and type of transfusion reactions (TRs) occurring in patients, reported to the blood bank at our institute.
Methods: A retrospective review of all TRs reported to the Blood Transfusion Institute of Vojvodina, between 2006 until the end of 2016 was done. All the TRs were evaluated in Blood Transfusion Institute of Vojvodina and classified using standard definitions.
Results: During the study period a total of 304,638 bloods and blood components were issued by our Institute. Out of the total 180 adverse reactions reported under the hemovigilance system, the most common type of reaction observed was febrile non-hemolytic transfusion reaction (FNHTR) 98 (54.4%) followed by allergic 69 (38.3%). Other less frequently observed reactions were hemolytic transfusion reaction 2 (1.11%) and other post transfusion reactions 11 (6.11%). Department of Internal medicine reported 133 patients with adverse reactions: a) department of Hematology 112 (54.6%) b) department for Dialysis 21 (10.4%). Products that caused adverse post transfusion reactions were: a) erythrocyte 62.4% b) fresh frozen plasma 11.2% and c) thrombocytes 14.4%
Summary/Conclusions: The frequency of TRs in our patients was found to be: 0.059% for erythrocyte (180 out of 304,638), 0.019% for thrombocytes and 0.010% for fresh frozen plasma. This can probably be an underestimation of the true incidence because of under reporting. Better coordination between transfusiologist and clinical doctors is necessary in treatment and monitoring of patients receiving multiple transfusions.

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SUCCESSFUL MANAGEMENT OF ABO MISMATCHED TRANSFUSION REACTION WITH PLASMA EXCHANGE

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Background: ABO mismatched transfusion cause serious complications such as anaphylactic shock, severe hemolytic reaction. If more than 30 ml of ABO mismatched blood is transfused, the patient could die. When detecting the situation of ABO mismatched blood transfusion, the transfusion should be immediately stopped.
Aims: For the treatment, we have tried plasma exchange with other medical treatment.
Methods: We tried plasma exchange with one plasma volume strategy and used albumin and fresh frozen plasma as replacement fluids.
Results: A 57 years old male patient presented with multiple trauma due to car accident. He had a deep laceration wound on his left neck and internal jugular vein were observed through the wound. And he also had open fracture on left posterior ankle with multiple fractures of both lower extremities and hip dislocation of right. His initial blood pressure and heart rates were 80/50 mmHg and 108/min. The initial hemoglobin/hematocrit was 13.3 g/dl/38.5% and they were dropped to 10.6 g/dl/31.2% in an hour. The transfusion of 2 units of red blood cell (PRC) were ordered and he was transferred to surgery room because he needed emergency operation for neck laceration. The patient was typed as O, Rh D positive, but a PRC of A, Rh A positive was transfused rapidly by mistake. When the accident was identified, the transfusion was stopped immediately, but after almost all of PRC was transfused. The patient showed hematuria in surgery room. The post-transfusion isoagglutinin

titer of Total/IgM anti-A were 1:1,024/1:512, respectively. The patient showed hematuria (occult blood 3+ on urinalysis), weakly positive on direct Coombs test and lactate dehydrogenase 1,313/IU/l. Plasma exchange was initiated immediately and medical treatment with high dose steroid with diuretics was done. The patient showed 1:8/less than 1:2 on isoagglutinin titer of Total/IgM anti-A respectively after first plasma exchange. Second plasma exchange was done and the patient looked recovered from acute adverse effect of transfusion, occult blood showed negative on urinalysis, and lactate dehydrogenase 718 IU/l. The plasma exchange was stopped and medical treatments for transfusion reactions were maintained for 10 days. The operations for his fracture were done and he was fully recovered.
Summary/Conclusions: Base on this case, the immediate treatment with plasma exchange with medical treatment is very helpful for ABO mismatched transfusion.

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HAEMOLYTIC DISEASE OF FOETUS AND NEWBORN (HDFN) AND HAEMOLYTIC TRANSFUSION REACTION DUE TO KIDD ANTIBODY IN HOSPITAL UMUM SARAWAK, MALAYSIA

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Background: Haemolytic Disease of Foetus and Newborn (HDFN) and Haemolytic Transfusion Reaction (HTR) may occur due to antibodies against Kidd antigen. In Malaysia, the prevalence of RBC alloimmunisation due to Kidd antibody for cases of HDFN and HTR have been reported however there is insufficient data in Hospital Umum Sarawak (HUS).
Aims: Therefore, the aim of this study is to determine whether Kidd alloimmunisation causes HDFN and HTR. Secondly, to determine the prevalence of Kidd phenotype among regular blood donors in HUS.
Methods: Records of alloimmunisation cases from 2011 to 2014 were retrieved and traced to the patients' medical records to determine whether Kidd antibodies is the underlying cause of HDFN and HTR in HUS. Secondly, to determine the prevalence of Kidd phenotype, two hundred and fifty (250) regular blood donors in HUS from 1st to 10th September 2015 were recruited. Blood samples were phenotyped for Kidd blood group using Diamed-ID gel card system.
Results: The results showed there were 1,109 cases of alloimmunisation recorded. Out of this 44 (4.0%) cases of alloimmunisation were due to Kidd antibody and 1,065 (96.0%) cases were due to other antibodies. Ten (10) out of 44 (22.7%) cases of alloimmunisation were due to Kidd antibody resulting in HDFN whilst 4 out of 44 cases (9.1%) resulting in HTR. These results were not statistically significant ($P > 0.05$). Meanwhile, the results of Kidd phenotype showed the presence of Jk (a+b+) phenotype in 110 out of 250 (44.0%) and Jk (a-b-) phenotype in 7 out of 250 (2.8%) blood donors. The other Kidd phenotypes detected were Jk (a+b) in 60 out of 250 (24.0%) and Jk (a-b+) in 73 out of 250 (29.2%) blood donors. Kidd phenotype was detected in four (4) ethnic groups; Chinese, 127 out of 250 (50.8%), Malays, 96 out of 250 (38.4%), Bidayuh, 25 out of 250 (10.0%) and Iban, 2 out of 250 (0.8%). The results also showed that Jk (a-b-) phenotype is present only in the Malays 7 out of 250 (2.8%) but not found in the other ethnic groups, and this is statistically significant ($P < 0.05$).
Summary/Conclusions: This study shows that alloimmunisation by Kidd blood group system is uncommon for the underlying HDFN and HTR in HUS. The most common Kidd phenotype among regular blood donors is Jk (a+b+). The prevalence of Jk(a-b-) phenotype in Malays in Sarawak is highest compared to earlier studies in Malaysia and Asia. In conclusion, there is low prevalence of Kidd antibody causing HDFN and HTR and Kidd blood group system was successfully characterised in regular blood donors in HUS.

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ANTI-CYTOKINE AUTOANTIBODIES AND RISK OF INFECTION IN HEALTHY BLOOD DONORS

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Background: Cytokine-specific auto-antibodies (c-aAb) may be found in healthy blood donors, and are known to be capable of suppressing the immune system through functional inhibition of their target cytokines. C-aAb are known to interfere with cytokine-based treatments of patients, and may be transferred through blood transfusions. Furthermore, c-aAb have been implicated in several immunodeficiency-related pathologies, and associated with opportunistic infections. However, c-aAb have also been linked to improved prognosis in autoimmune disorders, and are by some regarded as an intrinsic regulator of immune functions. Recently, c-aAb with cytokine function-potentiating capacity have been described. Despite their established immunomodulatory properties, the exact consequences of c-aAb remain incompletely understood.
Aims: The objectives of this study are (i) to study the presence of c-aAb in self-reported healthy blood donors, and (ii) to determine the impact of c-aAb on the immune system, as measured by donor history of infections.
Methods: C-aAb specific for interleukin (IL) -1α, IL-6, IL-10, Interferon (IFN) α, and granulocyte-macrophage colony-stimulating factor (GM-CSF) were previously detected in 8,972 healthy participants from The Danish Blood Donor Study (DBDS) using a microsphere assay for the Luminex 100 flow-cytometer. Measured c-aAb levels were then linked to information from the Danish National Prescription and Patient Registers available for all participants. We used logistic and cox regression analyses to evaluate the association of high c-aAb levels with increased risk of infection-related prescriptions or hospital diagnoses as compared with participants without high levels of c-aAb. Infections were defined in terms of infection-related prescriptions and diagnoses within 1 year of inclusion in DBDS, as well as the overall prescription and hospital histories of donors.
Results: We found correlations between high levels of c-aAb and altered subsequent infection risk, with the direction of the correlation depending on the c-aAb in question. High levels of c-aAb specific for the anti-inflammatory cytokine IL-10 was associated with reduced risk of both infection-related prescriptions and hospital diagnoses, compared to individuals with lower levels of IL-10 c-aAb (odds ratio = 0.43, 95% confidence Interval = 0.24;0.77). High levels of c-aAb specific for pro-inflammatory IFNα were significantly associated with increased risk of antimicrobial prescriptions following c-aAb measurement (odds ratio = 1.64, 95% confidence Interval = 1.07;2.51). With respect to hospital diagnoses none of the five investigated c-aAb were associated with risk of hospital admission with acute infections, yet we observed a significant positive association between higher levels of c-aAb for IL-6, and overall registered history of dermatological infections (odds ratio = 2.6, 95% confidence Interval = 1.34;5.07).
Summary/Conclusions: Naturally occurring c-aAb in healthy donors may inhibit regulatory components of the immune system, resulting in either increased or decreased risk of infection, depending on the cytokine in question. The exact immunoregulatory mechanisms of naturally occurring c-aAb in healthy donors and their potential effects in transfusion recipients require further studies.

Haemovigilance and transfusion safety

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DRUG USE AMONG BLOOD DONORS: IS THIS A PROBLEM?

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Background: Patterns of drug use in the general population have evolved greatly in recent years. At our blood center, deferral criteria for drug use are a few decades old and in need of revision. Data on drug use among blood donors are necessary to guide the development of relevant donor selection criteria.

Aims: To estimate the prevalence of illicit drug use and associated factors among blood donors and to compare it with the prevalence in the general population.
Methods: An anonymous online survey was carried out among individuals who successfully gave blood between 09/2015 and 06/2016 for which an email address was available. Participants completed a questionnaire assessing: (i) socio-demographic characteristics; (ii) use of illicit drugs in the previous 12 months; (iii) drug injection in the previous 12 months and lifetime. Prevalence rates were calculated according to age, sex, region of residence and first-time donor status. Overall prevalence rates were weighted according to the source population distribution of gender, age and first-time donor status. Logistic regression analysis was carried out to study correlates of drug use.
Results: Of the 35,850 donors contacted, 11,760 completed the survey (participation rate: 32.8%). Of them, 173 were excluded because of incomplete answers. Of the 11,587 participants, 47.4% were female vs 48.2% in source population (P = 0.12), 4.3% first-time donors vs 19.3% (P < 0.0001), and 11.8% <25 years old vs 22.2% (P < 0.0001). Rates of drug use in participants were: 9.4% (male: 8.7%, female 10.1%; P = 0.01; 18–24 years old: 31.2%, 25–49 y.o.: 12.2% and ≥50 y.o.: 3.0%; P < 0.0001). Based on weighted rates, 13.5% of blood donors reported any drug use. Weighted rates per type of drugs were: cannabis (12.7%); ecstasy (1.2%); hallucinogens (1.1%); amphetamines (1.0%); cocaine (1.1%); salvia (0.4%); GHB (0.2%); bath salts (0.1%); ketamine (0.1%); cannabinoids (0.03%); heroin (0.0%). Lifetime and past year drug injection were reported respectively by 0.1% and 0.01% of donors. A 2014–2015 survey of the general population (http://www.stat.gouv.qc.ca/statistiques/sante/etat-sante/sante-globale/sante-quebecois-2014-2015.pdf) indicated that the prevalence of drug use in the past 12 months was: cannabis (15.2%); cocaine (1.9%); amphetamines (1.3%); hallucinogens (0.9%); ecstasy (1.4%); ketamine (0.2%); (heroin 0.0%); (lifetime injection 0.8%). Multivariate analysis showed that factors significantly associated with drug use were: female gender [adjusted odds ratio (AOR): 0.76; 95% confidence interval (CI): 0.66–0.86], younger age (18–24 y.o. AOR: 15.21; CI: 12.55–18.44 and 25–49 y.o. AOR: 4.61; CI: 3.87–5.50) and living in a metropolitan region (AOR: 1.56; CI: 1.33–1.83). First-time blood donor status was neither confounding nor associated with drug use (AOR: 1.16; CI: 0.90–1.48).
Summary/Conclusions: Drug use prevalence among blood donors was similar to that of the general population and higher than expected. The impact of non injection drug use on blood safety is unknown. However, it is likely that our donor selection process is effective in excluding high risk drug users given our very low rates of transmissible disease markers. Despite being a deferral criterion, lifetime drug injection was reported by 0.1% of donors.

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RISKS AND BENEFITS FROM THE USE OF ELECTRONIC ISSUE

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Background: Electronic issue (EI) is selection of red cells where compatibility is dependent on the Laboratory Information Management System (LIMS) without the need to serologically crossmatch. EI depends on 4 main factors:
1. Having a fit for purpose IT system with appropriate algorithms that requires no manual intervention.
2. Current blood group is identical to the historical record and current antibody screen is negative (with no previously known irregular antibody) and results have been fully authorised on the LIMS.
3. No manual editing made to automated results.
4. Patient not excluded on clinical grounds.
Aims: SHOT data were reviewed to determine human errors resulting in inappropriate use of EI and to assess impact of EI on acute haemolytic transfusions reactions (AHTRs) due to antibodies to low frequency antigens (LFAs).
Methods: A retrospective analysis of cases (n = 432) where specific requirements were not met (SRNM) between 1/01/2012–31/12/2016 to distinguish where electronic issue was used inappropriately, and of severe AHTRs relating to LFAs.
Results: A total of 54/432 (13%) cases demonstrated specific requirements had not been met due to inappropriate use of EI. There were 54 cases where the patient should been excluded from EI. 26 where historical records containing important information were not heeded: 19 patients known to have clinically-significant red cell antibodies, 5 patients within 3 months of an with ABO-incompatible solid organ transplant, 3 patients with an ABO-incompatible haemopoietic stem cell transplant 28 were laboratory testing errors: 13 incorrect procedure followed, 8 antibody interpretation errors, 7 transcription errors In 5 years there were 2 serious AHTRs caused by antibodies to LFAs, (both anti-Wr^a) not present on screening cells. One death (2015) and another requiring resuscitation and admission to ITU (2012). Although anti-Wr^a is a well-recognised cause of HTR, reactions are rarely severe, with no reported deaths found in the literature.
Summary/Conclusions: Surveys by UK NEQAS for Blood Transfusion Laboratory Practice showed that EI is widely and increasingly used in the UK; 153/253 (60%) in 2016, up from 151/280 (54%) in 2012. Benefits include: timely provision of red cells for transfusion: reduction in red cell wastage; reduction in hands-on work, freeing staff to undertake other tasks and quality improvements. EI also allows remote issue of red cells using computer-controlled satellite fridges located closer to the patients. Automated pre-transfusion testing and result-transfer to the LIMS reduces the risk of transcription error. The main difficulty is managing patient records for acceptance for EI. This requires a human decision not controlled by the LIMS, and depends on correct clinical information being provided and updated. HTR due to antibodies to LFAs are an acknowledged, but small risk of EI, estimated at 1 in 500,000 to 1 in 1,000,000 transfusions (Garratty 2002). This possibility should always be considered when a patient develops an AHTR following transfusion. Retrospective crossmatch should be undertaken to confirm the presence of a red cell antibody. Patients with these antibodies should be flagged as being unsuitable for EI, to prevent future AHTR.

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INCIDENCE OF CMV INFECTION IN CMV-NEGATIVE RECIPIENTS OF CMV-NEGATIVE HEMOPOIETIC STEM CELLS SINCE THE CHANGE OF TRANSFUSION POLICY TO UNSCREENED BLOOD PRODUCTS

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Background: Cytomegalovirus (CMV) infection can cause major morbidity and mortality in stem cell transplantation. Primary CMV infection may follow infusion of stem cells, blood products from CMV seropositive donors, as well as occurring via direct contact with individuals with active infection. In October 2012 SaBTO recommended that leukodepletion be considered an adequate method for prevention of transfusion-transmitted CMV in hemopoietic Stem Cell Transplant (SCT) recipients
Aims: To establish CMV infection rate with the use of CMV unscreened, leukocyte-reduced blood components, transfused to CMV seronegative recipients of allogenic stem cells from CMV-negative donors.
Methods: Retrospective analysis of incidence of CMV infection in neg/neg allogenic transplant recipients, transfused with CMV unscreened, leukocyte-reduced blood components at Kings College hospital, London, United Kingdom
Results: 72 (49 males, 23 females) patients received CMV neg/neg transplants between January 2013 to January 2016. The mean age was 53 (Range 20–76) years. The median follow up was 15 months; 70 (97%) patients were alive at day +100, and 54 (75%) patients were alive at follow up in May 2016. The stem cell source was peripheral blood in all 72 patients. Sixty nine of the 72 patients (95.8%) received a T-depleted graft, with Alemtuzumab in 66.6% and ATG in 33.3%. Only 3 patients had T cell replete transplants (4%) median number of CMV PCR tests done post-transplant was 33; during D + 100 days post-transplant, the median number of tests were 18. one patient was found to have positive CMV PCR findings with in D + 100. The patient received two apheresis granulocyte units from a CMV IgG positive donor under concessionary release. Over the study period, a total of 1,329 leukocyte-reduced components were transfused to 72 patients, comprising 601 red cell units, and 729 platelet units, and 13 apheresis granulocytes. Of the platelet units, 435 were obtained by single donor apheresis and 294 were pooled from four whole blood donations. The 1,329 components therefore correspond to 2,212 donor exposure. During the first 100 days post transplantation, 708 components were transfused, equating 1,158 donor exposure
Summary/Conclusions: 1/72 patients had transfusion-transmitted CMV infection over a 3 year period; this patient received non-leukodepleted blood products from a CMV-positive donor. Our findings support leukocyte reduction as a safe strategy to prevent transfusion transmitted CMV infection in allogenic SCT recipients

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CELL SALVAGE INCIDENT REPORTING – THE UK EXPERIENCE

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Background: Cell salvage is an integral part of Patient Blood Management and when used correctly and safely it effectively reduces the use of allogeneic transfusion in surgical patients. The basic process remains the transfusion of human blood and therefore needs to be monitored in the same way as other transfusions. The UK haemovigilance scheme, SHOT (Serious Hazards of Transfusion), has been collating incidents relating to autologous transfusion since 2008, with electronic reporting since 2010.
Aims: To review the UK experience of incident reports relating to cell salvage.
Methods: SHOT databases, from 2010 to 2016, were interrogated to capture all incidents relating to autologous transfusion. Reports were categorised by acuity, surgical specialty, incident type and outcome.
Results: 125 incidents were reported, of which 124 related to cell salvage. It appears that the practice of predeposit and acute normovolaemic haemodilution has almost completely disappeared from clinical practice in the UK. The largest number of incidents (42) was reported in 2011 with the smallest number in 2016 (9). The majority of incidents occurred in elective surgery (102), with orthopaedic procedures being the highest reported category (60). The type of cell salvage used was predominately intra-operative cell salvage (ICS, 78), with 42 incidents in post-operative procedures (PCS) and 2 peri-operative (combined). In PCS, 24 adverse events (1 device failure, 23 human errors) and 18 adverse reactions (1 hypotension, 14 pyrexia/rigors, 3 other) resulted in 15 minor morbidities. In ICS there were 47 adverse events (18 device failures, 26 human errors, 3 other) and 33 adverse reactions (27 hypotension, 1 pyrexia/rigors, 5 other), leading to 26 minor and 7 major morbidities including 2 deaths not attributed to cell salvage. In the 27 ICS hypotensive incidents, 20 occurred with the use of a leucocyte depletion filter with citrate the anticoagulant on 18 occasions.
Summary/Conclusions: Cell salvage is an invasive intervention that requires adequate standards, training and competency assessment to avoid unnecessary adverse events. Of particular note was the very high percentage of adverse events related to human error and human factors highlighting the need for adequate training and ongoing assessment of competency. Adverse reactions are by definition unforeseen, but data trends show consistent themes for the types of cell salvage used. The most harmful reaction appears to be sudden onset severe hypotension. Further work is needed to elucidate the mechanism by which this occurs. We cautiously endorse the safety of cell salvage but recognise that many adverse events may be unrecognised or not reported.

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REVIEW OF NATIONAL HAEMOVIGILANCE REPORTS AS PART OF A PLASMA PATHOGEN INACTIVATION SYSTEM POST-MARKET SURVEILLANCE PROGRAM

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Background: National Haemovigilance (HV) Systems are an important tool for guaranteeing safety of patients, donors and transfusion service personnel, improving quality of blood products and promoting their optimal use in clinics. National HV reports are published on a regular basis by transfusion services, and provide data that can help medical device manufacturers complete their product-related safety data. In our organization, a review of multinational HV data is performed annually as a part of the post-market surveillance program. Here, we provide a summary of last results for Macopharma's pathogen inactivation (PI) system designed to reduce viruses and bacteria in plasma.
Aims: In order to establish a complete safety and performance profile for our THERAFLEX MB-Plasma system, we collected and analyzed data from peer reviewed journals as well as from sources which might report negative outcomes. Haemovigilance reports are supposed to focus mainly on adverse events and therefore meet this criteria perfectly. Moreover, they contain nationwide data from large patient populations and are a valuable tool for the identification of rare adverse events.
Methods: For data collection, we identified countries, where the THERAFLEX MB-Plasma system or equivalent procedures using methylene blue (MB) in combination with visible light have been in use and national HV reports and/or publications with HV data are available. These reports were reviewed and relevant data were extracted for further analysis. In addition, we screened journal articles that might contain HV data, using PubMed and accessing transfusion journals directly. Some important numbers were taken from national transfusion service activity reports. This review was completed with results from a recent international, multicentric, prospective phase IV clinical study (NCT02007473) conducted by Macopharma in partnership with a globally recognised CRO from 2013 to 2015.
Results: 41 HV reports and related documents (activity reports, official letters) and 21 publications containing HV data from clinical studies were identified and reviewed. The collected data related to the following countries: France, Belgium, Spain, Italy, the UK, Greece and Austria. The covered period was from 2007 and up to 2015, depending on the availability of HV reports and duration of use of the PI system. For equivalent methods, we reviewed HV and clinical data from 1992 onwards. During the analyzed period for THERAFLEX MB-Plasma and other PI systems, there was no indication from any country about an increased number of adverse reactions to MB-plasma compared to other types of plasma, with the only exception of France during 2008–2012. There was no evidence for an increased plasma volume transfused with MB-treatment.
Summary/Conclusions: This review of HV publications provided additional clinical data confirming the clinical efficacy and a good safety profile of THERAFLEX MB-Plasma system in terms of plasma transfusion-related adverse events and, particularly, adverse reactions.

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TRANSFUSION ERRORS IN TRANSPLANTATION

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Background: Patients who receive allo or auto haemopoietic stem cell transplants (HSCT) or solid organ transplants have specific transfusion requirements such as the need for irradiation of cellular components, and for allografts (haemopoietic or solid organ), in the UK from March 2016, HEV-screened components. Where a HSCT allograft is from a donor of different ABO and/or D type the transfusion requirements are more complex and change with time over the transplant period requiring close collaboration between the transplant unit and transfusion laboratory.
Aims: To review and categorise transfusion errors in stem cell and solid organ transplant patients.
Methods: To review and categorise transfusion errors in stem cell and solid organ transplant patients.
Results: 298 cases were identified. ABO and D errors accounted for 105/29 (35.2%) cases, including 51/105 ABO-mismatches and 19/105 D-mismatches resulting in wrong transfusions, with the remaining 35/105 cases being near misses, i.e. the ABO or D error was detected before any component was transfused. HSCT patients were affected in 200/298 (67.1%) and solid organ recipients in 99/298 (33.22%) (1 patient had both HSCT and solid organ transplants). There were 26 ABO-incompatible red cell transfusions to HSCT allograft recipients, with 1 serious haemolytic transfusion reaction. Transplant transfusion errors resulted mainly from poor communication, such as failure of the transplant team to inform the transfusion laboratory that an allograft was taking place (183/298, 61.4%) and failure to update or heed flags on the laboratory information management system (LIMS) (84/298, 28.2%). Other errors included poor clinical decision-making or misunderstanding (31/298, 10.4%). The recommendation for irradiated components for patients receiving solid organ transplants has been disputed: some transplant centres have opted not to follow national guidelines, but do not communicate this to the patient's local hospital where the standard guidance may be followed, leading to confusion.
Summary/Conclusions: It is essential that clinical and transfusion laboratory teams are in close communication to ensure optimal component provision, in particular to prevent ABO incompatible transfusions to HSCT patients. It is also vital that the LIMS is kept updated and used effectively. Further training and education of both clinical and laboratory staff may be required to ensure full understanding of the complexities of transfusion to transplant recipients. There is surprisingly little guidance available for transfusion of transplant recipients, particularly for ABO-incompatible solid organ transplants, and SHOT recommends the development of national guidelines.

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SYPHILIS SEROCONVERSIONS AND OTHER ALERTS FROM THE BLOOD CENTRE: HOW DOES THE HOSPITAL TRANSFUSION SERVICE ADDRESS THE LOOK BACK REQUIREMENTS?

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Background: The Regional Blood Centres in our country are responsible for the analysis of blood donations. When an infectious marker turns positive in a previously seronegative donor, the Transfusion Service is informed in order to perform a retrospective study of the outcome of previous donations.
Aims: To analyse the performance of a Transfusion Service in response to the requests from the Blood Centre and calculate: the turnaround time from the alert to the transfusion service reply, the test results obtained in the recipients and the traceability of the donations.
Methods: We analysed the e-mail alerts from the Blood Centre that were consecutively registered in the Transfusion Service of a 400-bed hospital from 2011 to 2016. After an alert for recall, we immediately tried to retrieve the blood unit. In the case of a look back, we searched our electronic records. When the recipient was found not alive, we notified the date and cause of exitus to the Blood Centre. In the case of a living recipient, we notified the responsible physician for him to request the appropriate serology. We communicated the serologic results via e-mail to the Blood Centre. We classified the alerts and calculated the time elapsed from the e-mail alert to the closing e-mail answer.
Results: In 6 years, 60.531 blood components were transfused and 21 alerts were received from the Blood Centre: 18 requests for recipient look back and 3 requests for withdrawal of components. No withdrawal was possible since all components had already been transfused (a platelet donation made the day before the onset of herpes zoster, two distributed donations of female plasma, and a plasma unit from a donor who seroconverted to syphilis). The 18 recipients were 15 men and 3 women: 50% from the Haematology Department, and the rest from ICU, Emergency, Surgery, Gastrointestinal Diseases, and Neurology Departments. In 2 cases, the component had been transfused in another hospital. All blood components originated from repeat donors. The look back was related to syphilis (n = 6), 33.3%, HIV (n = 6) (33.3%), HCV (n = 2) (16.6%) and HBV (n = 3) (16.6%). The implicated components were platelet pools (50%), red blood cells (40%) and plasma units (10%). By electronic search of patient notes, we found out that 9 patients had died and 9 were still under control: 6 in Haematology, 2 in Gastrointestinal Department, 1 in General Surgery. Once informed, the physicians in charge obtained the appropriate serological tests. The results were negative in all cases (3 for syphilis, 3 for HIV, 2 for HBV and 1 for HCV). The traceability was electronic in 20 notifications and in paper in the remaining case. The turnaround time was an average of 25 days (range 1–102 days) for our cases and 37 days (range 1–120 days) if we included patients transfused in another hospital.
Summary/Conclusions: In the absence of comparable data, our computer application allows a quick response to the alerts from the Blood Centre. We need to develop common guidelines on the information patient should receive about look back procedures. The frequency with which these infectious alerts appear is a cause for concern. In our opinion, these data should motivate a review of donor education policies, donor information, and their commitment to safe donation.

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LESSONS LEARNED FROM NEAR-MISS AND DONOR SAFETY REPORTS IN BLOOD TRANSFUSION CENTRE OF IZOLA (SLOVENIA) IN THE PERIOD 2002–2016

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Background: Blood transfusion center of Izola (CTD IZ) is part of the Blood Transfusion Centre of Slovenia (major blood establishment in the country). It is located in the south-western part of Slovenia. CTD IZ has a function of blood establishment (blood collection) and hospital blood bank (cross matching, blood storage and haemovigilance). CTD IZ covers 4 hospitals in the region (General hospital Izola, Orthopaedic hospital Valdoltra, Cardiovascular hospital Medicor and Hospital Sezana) and primary health centers in 5 towns. CTD IZ collects approximately 5,300 blood units, issues 5,500 units and performs 19,000 imunohaematological tastings per year. Haemovigilance in CTD IZ was established as an organized entity in 1996. In 2002, when a Slovenian haemovigilance service was formally established, CTD IZ conformed to the Slovenian and IHN guidelines.
Aims: Near-miss reports can provide us with the information how vigilant the system supporting hospital blood banks is, is the staff involved in blood transfusion trained and alert enough, is the IT support good enough and finally what can we do to improve the blood transfusion system. Donor safety is a major concern of any blood establishment in a process of ensuring safe and qualitative blood components. Blood donor will return to blood establishment only if he remains safe, satisfied and healthy. Safety in blood donors has always been of a major concern in CTD IZ so always before a donation a physician checks him up (a trend in blood analysis is done, sometimes additional blood analysis are performed, if a trend in lowering haemoglobin is observed a deferral of larger interval between donations is advised to a donor or a check-up at the general practitioner is suggested).
Methods: Oversight and management of data collected during the period is constantly performed by the staff in CTD IZ. Blood transfusion informational system Datec provided part of the data (managing blood donors); near-misses were collected on spread-sheets prepared by national haemovigilance service. Data analysis was done using a simple statistic calculation.
Results: In the 2016 around 25% (12/47) of overall near-misses were due to laboratory errors. Wrong patient identification, WBIT and wrong order form were responsible for the remaining near-misses. In the past years we became more vigilant towards near-misses and blood donors' adverse events. To lower the near-miss events the IT connection between hospitals and hospital blood banks is essential. There has been an almost continuous coordination in the mentioned field for the last 15 years but no effective results have been achieved. At the moment hospitals still order blood components and laboratory tests on paper forms. According to the statistical analysis the number of adverse events in blood donors is increasing (from 40 in 2012 to 100 in 2016) probably due to the better definitions of adverse events and higher vigilance among the staff. The number of severe adverse events is relatively low and it is not increasing.
Summary/Conclusions: We can conclude that both reports gave us a major insight into the status of laboratory management, communication with the clinical departments in the hospitals, as well as blood donor management within our center.

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KOREAN HEMOVIGILANCE SYSTEM – ANNUAL REPORT OF 2016

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Background: We have set up a national hemovigilance system supported by the Korean Ministry of Health and Welfare but independently operated by the Korean Society of Blood Transfusion (KSBT). We have operated the system for 9 years including a two-year pilot study.
Aims: We report the results of the ninth year's operation of the Korean hemovigilance system.
Methods: We continued to operate the national hemovigilance system. We have amended the classification of transfusion reactions and modified the reporting form according to the revised classification. We have also upgraded the homepage of the Korean hemovigilance system (www.kohevis.or.kr) and held an explanatory meeting for participating institutions.
Results: The Korean hemovigilance system is a voluntary reporting system and all adverse events including transfusion reactions and incidents are reported. A total of 215 hospitals participated and 169 hospitals reported 3,375 adverse events in 2016. Among these, 60 hospitals reported zero during the year. The numbers of participating hospitals and adverse events have increased every year and the 169 reporting hospitals accounted for about 74.2 of transfusions in Korea. There were 86 reports of incidents. A half of the incidents were related to blood sampling and about a fifth (19.8%) occurred during transfusion in the ward. Overall, 3,293 adverse transfusion reactions were reported. Febrile non-hemolytic transfusion reaction (1,783, 54.1%) and allergic reaction (821, 24.9%) account for most adverse reactions. Five cases (0.2%) of acute hemolytic transfusion reaction, 3 cases (0.1%) of delayed hemolytic transfusion reaction, and 1 case of transfusion-related acute lung injury were reported. After 9 years of operation (including a 2-year pilot study), a total of 11,842 adverse events have been reported to the hemovigilance system.

Summary/Conclusions: We have operated the system successfully for 9 years and it seems to work very effectively. Data from the hemovigilance system are expected to support the development of blood safety strategies in Korea.

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WHAT HAPPENED TO THE HIV INFECTED PATIENTS WITH THALASSAEMIA SYNDROMES IN GREECE? A 30-YEAR MULTICENTER STUDY

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Background: Transfusion Transmitted Infections (TTIs) are among the major drawbacks of transfusion particularly in patients with thalassaemia who are exposed to chronic transfusion. We focus on TT-HIV infection in thalassaemia, first diagnosed in a patient in 1982 followed by two infections in 1983 and one in 1984. Donor blood is screened for HIV antibodies since September 1985 and all the 3000 thalassaemic patients were tested by the end of 1987. Surveillance for the detection of seroconversion and follow up of infected patients has been performed by the treatment units and reported to the Haemovigilance Centre.
Aims: We present data on the survival of HIV infected thalassaemic patients in 1987–1994 and 1995–2015 along with national epidemiological data on donor blood.
Methods: About three thousand multitransfused patients (3–65 years old, 45% splenectomised) were screened for anti-HIV antibodies by ELISA and CHLIA serological assays. Positive samples were confirmed with Western Blot. Individual NAT testing of HIV-RNA was implemented in 2008. Demographic data (age, sex, region), blood units transfused, splenectomy, iron chelation therapy, serum ferritin and antiretroviral treatment (ART) were recorded.
Results: In 1982–2015, 43 thalassaemic patients tested positive for HIV infection.
Forty-two patients who had received 819,000 units of RBCs (1:19.500) were infected by the end of 1987. Anti-HIV prevalence in blood donors was 0.002%. TT-HIV infection risk was reduced significantly by 2005 with seroconversion in three patients transfused with seronegative blood donated from three different donors during the serologically silent window period. Therefore the residual risk of TT-HIV infection in these patients 1988–2015 was estimated at 1:290,666 serologically screened blood units. The corresponding figure in the blood donor population was 1:1,833,333 blood units. No case of TT-HIV has been recorded since implementation of NAT screening (August 2008–2015).
Of the 32 patients (74%) who died up to 2015, 26 (81%) progressed to AIDS at a mean age of 16.5 ± 9.2 years. AIDS occurred mainly in CDC stages B1 and C1 of the disease (main causes were diseases of the central nervous system, pneumonia carinii, cryptococcal meningitis and lymphoma) while the remaining deaths were mainly due to cardiac failure. One deceased patient (cardiac failure) was co-infected with HBV and HCV. Hepatitis B and C prevalences were higher in HIV seropositive patients than in seronegative.
Of the 13 survivors (mean age 42 ± 7.1 years), two have history of splenectomy and two are anti-HCV positive with PCR HCV-RNA negative. Most patients have negative viral load and are free of HIV symptoms.
A multivariate analysis of data in the overall period demonstrates that serum ferritin levels are statistically significantly associated with the duration of survival after diagnosis of HIV infection in this group. This is attributed to the anti-oxidant activity of high doses of desferrioxamine and deferiprone or deferasirox and its relationship with the progression of HIV disease.
Summary/Conclusions: This study demonstrates the success of blood safety against HIV infection and the value of iron chelation therapy as well as the importance of ART of survival and wellbeing of patients with thalassaemia syndromes.

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REVIEW OF NATIONAL HAEMOVIGILANCE REPORTS AS A PART OF A PLATELET ADDITIVE SOLUTIONS POST-MARKET SURVEILLANCE PROGRAM

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Background: National Haemovigilance (HV) Systems play an important role for guaranteeing safety of patients, donors and transfusion service personnel, while improving quality of blood products and technologies and optimizing their clinical use. National HV reports are published on a regular basis and provide data that can help medical device manufacturers to complete their product safety- data base and to meet regulatory requirements for post-market surveillance (PMS). A review of multinational HV data can be also interesting for transfusion specialists. This review deals with HV data on platelets suspended in platelet additive solutions (PAS). Macopharma is the manufacturer of SSP (PAS II, PAS-B) and SSP+ (PAS IIIM, PAS-E) platelet additive solution.
Aims: In order to establish a complete safety and performance profile for our platelet additive solutions, we collected and analyzed data not only from peer reviewed journals, but also from sources which might report negative results. Haemovigilance reports are supposed to focus mainly on problematic issues and therefore meet these criteria perfectly. Moreover, they contain nationwide data from large patient population and are a valuable tool for identification of rare adverse events.
Methods: For data collection, we identified countries, where Macopharma SSP and/or SSP+ solution is marketed and national HV reports and/or publications with HV data are available. Those reports were reviewed and relevant data were extracted for further qualitative and quantitative analysis. In addition, we screened peer reviewed journal articles that might contain HV data, using PubMed and accessing transfusion journals directly. We compared results with data from two randomized clinical studies with PAS from the past. Some important numbers were taken from national transfusion service activity reports.
Results: 82 HV and related reports and 3 publications containing HV data from clinical studies were identified and reviewed. The collected data related to the following countries: Australia, Austria, Belgium, Denmark, France, Germany, Ireland, Italy, New Zealand, Russia, Spain, Sweden, UK, and Switzerland. The covered period was from 2007 until 2015, depending on the availability of HV reports and the beginning of PAS usage. In New Zealand, Switzerland and the UK, the introduction of PAS significantly reduced the rate of adverse (primarily allergic) reactions related to platelet transfusions. Furthermore, a prospective clinical study in a paediatric haematology hospital in Moscow demonstrated a significant reduction of non-haemolitic febrile transfusion reactions for platelets prepared in SSP+ compared to those suspended in plasma. No adverse reaction related to PAS was reported in any report or publication. One bacterial transmission through a platelet concentrate in SSP+ was recorded in Austria in 2015. However, further investigations excluded the PAS as a cause of bacterial contamination.
Summary/Conclusions: National HV reports are a valuable source of safety data not only for transfusion services, but also for manufacturers for the PMS program. This review of HV publications provided additional clinical data confirming a good safety profile of platelets in additive solutions. The replacement of plasma with PAS, including Macopharma SSP and SSP+, results in fewer adverse transfusion reactions.

P-696

CONSENT TO, AND REFUSAL OF BLOOD AND BLOOD PRODUCTS: MEDICO-LEGAL ASPECTS

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Background: It has been well documented that consent to blood and blood products is a crucial step in safe transfusion practice. Informed consent involves a number of steps. Firstly, a clinician explains the indications for transfusion, followed by risks and benefits, and finally any possible alternatives to transfusion that might be available. To date, local policy has only provided guidance on how to seek consent from patients for blood and blood product transfusions. Thus, patients were not provided with alternatives to transfusion that may be available. Clinicians at the bedside are often challenged when providing patients with alternatives to transfusion where the patient refuses blood and blood products.
Aims: Research into the area of consent and refusal of blood and blood products aimed to provide clear guidance as to the consent process for blood and blood

products, as well as a framework for clinicians to use when a patient refuses blood transfusions.
Methods: Following a comprehensive review of the literature on consent and refusal of blood and blood products; state and national legislation on consent; medical and nursing feedback into the consent process; as well as consumer input, a framework was created to enable clinicians to both ensure that consent was well informed, and in cases where a patient refused a transfusion, alternative options were made available to the patient.
Results: The framework created was founded on evidence based national patient blood management guidelines, which aims to optimise a patient before elective surgery to mitigate the necessity for transfusions. This also allows patients to opt to receive certain blood products if it aligned with either religious or personal beliefs. The framework created was in the form of a transfusion refusal guidance policy, and refusal of blood and blood products form. This addressed the needs of patients presenting to hospitals in the local health district, many of whom -are of Jehovah's Witness faith, and may decline transfusion of certain blood products.
Summary/Conclusions: This new framework ensures that clinicians at the bedside have easy access to information on both consent to, and refusal of blood and blood products, to deliver safe and effective patient care.

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Abstract has been withdrawn.

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Abstract has been withdrawn.

P-699

THE FIRST PRELIMINARY STEP TOWARD NATIONAL HEMOVIGILANCE IN LEBANON

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Background: Reporting and analyzing adverse transfusion reactions (ATR) is an integral part of the Hemovigilance surveillance system aimed at improving the safety and quality of Blood transfusion. In Lebanon, a well-established hemovigilance system is lacking and thus, there are neither available national data nor official published reports regarding ATR or any other aspect of blood transfusion; and most of the hospitals do not have an efficient or standardized monitoring and reporting systems. A recent national legislation for reporting ATR was set in 2016 but still not yet implemented.
Aims: The present study was undertaken to determine the frequency and type of ATR occurring in hospitalized patients reported to the blood bank at a major tertiary care health center. Since the concept of Hemovigilance is newly diffused in Lebanon, such work may highly contributes toward promoting national Hemovigilance.
Methods: This is a retrospective review of all ATR that were reported to the Department of Transfusion Medicine at Sacre-Coeur hospital, between January 2003 and January 2012, date of which universal leukoreduction was introduced. All ATR were clinically evaluated by the blood bank physician and classified in accordance with the standards and recognized definitions defined by American Association of Blood Banks.
Results: During the study period, a total of 22,854 of non leukoreduced Blood and Blood components were transfused of which 102 ATR were reported to the blood bank. The majority of patients who experienced an ATR were female (60%). Febrile non hemolytic transfusion reactions (FNHTR) were the most frequent ATR followed by allergic reactions (57% and 26% respectively). Less frequently observed reactions were Hypervolemia (9%), Anaphylaxis (4%), Transfusion related acute lung injury (1%), Citrate toxicity (1%) and Hypotension secondary to Angiotensin Converting Enzyme inhibition (1%). Packed red blood cells, which accounted for 79.6% of the transfused blood components, were responsible for 92% of the ATR. No alloimmunization was reported since alloantibodies screening post transfusion is not mandatory in our country.
Summary/Conclusions: The frequency of ATR per non leukoreduced blood and blood components was found to be 0.45%, which is closed to the rate reported by several international well organized Hemovigilance systems. However, the true incidence may be underestimated, that's why both fully functional hospital transfusion committee and education/awareness campaigns for health care workers are needed to improve the reporting system. The most common reaction observed was FNHTR followed by allergic reactions. The fact that no alloimmunization was reported highlights the need to implement regular antibody screening after transfusion.
This study represents the first published report regarding ATR in Lebanon and a model of an institutional Hemovigilance effort that can be followed. It would be interesting to use these raw data, in combination with other health care centers data to assess the impact of universal leukoreduction introduced in 2012. There is an urgent need in Lebanon to implement the national blood policy along a centralized Hemovigilance system and to activate the data collection mechanism using standardized tools, which will help in organizing and improving the safety of transfusion services.

P-700

Abstract has been withdrawn.

P-701

GLUCOSE 6-PHOSPHATE DEHYDROGENASE DEFICIENCY SCREENING IN THAI BLOOD DONOR

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Background: Glucose-6-phosphate dehydrogenase is a metabolic enzyme that controls oxidative stress in red blood cells. G-6-PD deficiency is also important in blood transfusion because red blood cells from donor with G-6-PD deficiency may result in a hemolytic reaction in patients. In high prevalence areas, it is found that transfuse red blood cell with G-6-PD deficiency is a risk factor of severe hemolytic transfusion and transfusion of the screening for G-6-PD deficiency in all red blood cell is recommended.
Aims: To assess the prevalence of G-6-PD deficiency and study G-6-PD activity in blood donors from National Blood Centre, Thai Red Cross Society.
Methods: a total 330 cases of blood donors were collected from National Blood Centre, Thai Red Cross Society. All samples were analyzed by using fluorescent spot test (FS test) for G-6-PD deficiency testing and using biochemical tests for testing the G-6-PD activity.
Results: The prevalence G-6-PD deficiency in blood donor was found in 4.2% (14/330) which male was higher G-6-PD deficiency prevalence than female (7.9% and 0.6% respectively). Then, we compared the G-6-PD activity in G-6-PD deficiency group and normal group. We found that G-6-PD activity in G-6-PD deficiency group was significantly lower than normal group (0.45 ± 0.07 IU/gHb vs 8.76 ± 2.60 IU/gHb). Moreover, the G-6-PD activity in male blood donors was lower than female blood donors (8.22 ± 2.56 IU/gHb, 9.38 ± 2.63 IU/gHb respectively).
Summary/Conclusions: Our finding showed 4.2% blood donors were severe G-6-PD deficiency particularly in male blood donors. Thus, the transfusion of red blood cells with G-6-PD deficiency was increased a high risk factor in hemolytic transfusion process, particularly in infants. Therefore, the G-6-PD deficiency screening should be considered to test in blood donors before handling in patients.

P-702

THE RETROSPECTIVE EXPLORATION OF POLYMORPHISM DISTRIBUTION OF BLOOD DONORS' MILTENBERGER BLOOD TYPE AND GP.MUR BLOOD CLINICAL INFUSION

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Background: Sub system of MNS blood group system is the Miltenberger system, gp.mur is a version of the system, the crowd in Europe is rare, the clinical significance of small, but in some Eastern populations in the distribution of high frequency, it has been shown that the anti -mur antibody can cause hemolytic transfusion reaction (HTR) and hemolytic disease of the newborn (HDN). At present, China has not yet been a routine screening of clinical blood transfusion before Mur antigen, clinical effect and so on gp.mur polymorphism distribution of the Miltenberger blood group system to improve the safety of clinical blood transfusion in China has important significance.
Aims: To establish a simple and rapid detection method of Miltenberger blood group system, and investigate the polymorphism distribution of Miltenberger blood group system to donate blood. For gp.mur positive blood patients were retrospectively reviewed. The clinical significance of gp.mur positive blood.
Methods: According to the molecular basis of Miltenberger blood group system, specific primers were designed using polymerase chain reaction sequence specific primer (PCR-SSP) screening and sequencing, molecular biology detection method of pcr-ssp- sequencing to establish the Miltenberger blood group system. Serum and verify accuracy of the method using anti -mur standard. A retrospective survey of gp.mur positive blood transfusion in blood donors from 2011–2013, Miltenberger blood group identification and anti-Mur antibody testing and clinical transfusion effect analysis.
Results: This study successfully established the molecular biological detection method of pcr-ssp- sequencing of Miltenberger blood group system. Serological methods to verify fully comply with
This study successfully established the molecular biological detection method of pcr-ssp- sequencing of Miltenberger blood group system. Serological methods to verify fully comply with. Methods 24 cases of Miltenberger blood group positive individuals were screened by PCR-SSP from the voluntary blood donors in Henan nationality, accounting for 0.7%.12 patients with gp.mur positive blood transfusion were observed and followed up, of which there were male and female in 4 cases, and the Miltenberger blood group was negative for gp.mur. The sera of patients with anti -mur antibody were 2 and 4, respectively .
Summary/Conclusions: Group Miltenberger blood group system detection blood donors of Han nationality in Henan Province, e gp.mur phenotype, the frequency of 0.9%. Infusion of gp.mur positive red blood cells of the patients developed anti -mur antibody, can cause transfusion reactions, recommend routine screening before blood transfusion of gp.mur blood group.

P-703

PRESCRIPTION OF PLATELETS CONCENTRATE DURING A DENGUE FEVER EPIDEMIC OUTBREAK: CASE OF BURKINA FASO IN 2016

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Background: Dengue is an infectious disease with a recurring incidence, especially in developing countries.
Burkina Faso is facing more or less significant outbreaks of dengue fever with varying clinical profiles since 2013. Nevertheless, each outbreak is accompanied by an increase in platelets consumption. This raises the question of the rational use of blood in our context characterized by a chronic shortage in blood products and insufficient transfusion safety.
Aims: The aim of the study was to assess the request and delivery of platelets concentrates in dengue fever outbreak in Burkina Faso.
Methods: We conducted a cross-sectional study that included patients with AgNS1 and/or IgM or IgG positive tests.
Results: Around 1,300 (21%) out of 6,200 cases of dengue (likely cases) have been hospitalized in public (29.3%) and private hospitals (70.7%). A prescription of platelet concentrates (PCs) has been issued for 184 (14.1%) patients. The sex-ratio (M/F) was 1.5 and the average age 37.3 ± 18.1 years. A total of 220 prescriptions of PCs have been issued for the dengue patients. Hemorrhagic symptoms were reported in 40%. According to platelets count, severe to high risk of hemorrhage were reported in 52.3% of cases from whom hemorrhage was effectively present in 47 cases.
The need in PCs units for the 184 dengue patients represented 33.8% (1,243/3,677) of the total PCs units ordered in 2016. During the outbreak period, the proportion of PCs units requested for dengue patients represented 59.9% of PCs units ordered. The satisfaction rate was 60.5% (752/1,243). The average delivery time was 1.14 days.
Summary/Conclusions: Transfusions of platelets concentrate were used as part of the treatment of dengue fever manifestations. In most cases, the prescription of platelets was not justified. In our context characterized by blood products shortage and due to the delicacy of PCs, the blood transfusion service has had to increase his platelets production to meet the hospitals needs.
Some of the clinical manifestations may be related to unfavorable outcomes.

P-704

ABO BLOOD TYPE REGISTRATION CAMPAIGN OF NEW PATIENT IN SEVERANCE HOSPITAL

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Background: Blood transfusion accidents caused by incompatible ABO blood type can be observed from the UK's SHOT or Japan's report continuously. Recently, one case was reported in Korea. Severance Hospital is conducting blood type test using automated equipment. Since the procedure for checking the blood components is performed by two medical staff, there is a high possibility of an error in blood collection for crossmatching.
Aims: To prevent ABO incompatible blood transfusion accidents due to blood sampling errors, we conducted a campaign to perform at least two ABO type tests at different times. It is for the purpose of completing safe blood transfusion by carrying out improvement activities to eliminate ABO blood type incompatible blood transfusion at Severance Hospital.
Methods: Before the campaign, we informed the start of the "ABO blood type registration campaign of new patient" through two in-house newsletters. The Order Communication System (OCS) program was revised so that the ABO blood type is displayed always in OCS, so blood type tests for patients without blood type are prescribed. A popup window was created to emphasize once again the precautions to collect blood samples for transfusion. Hospital blood transfusion committee requested cooperation to add ABO blood type tests to the pre-hospital screening of patients expected to undergo surgery. Fourth edition of transfusion guidelines were sent to each medical staff to educate them about prevention of ABO blood type incompatible transfusion and to educate them about the necessity of two blood type tests before transfusion. At Blood bank, if there was no previous ABO blood type, the ABO blood type test prescription was induced.
Results: At Severance Hospital, among the patients who were prepared for blood transfusion, the number of patients without blood type test records increased from 35% in June to 39% in December. But blood type tests for double confirmation after admission increased from 19% to 56%.
Summary/Conclusions: In early 2016, two of five blood collection errors that occurred in the first blood transfusion patients. One case was different from the blood type that the patient knew. One other case was found from a blood sampling error in the patient who already knew the blood type. Blood type test before admission has not been performed yet (35%, 39%). But after admission, ABO blood type was double confirmed before blood transfusion through improvement activities in the medical staff and the Blood bank (19%, 56%). While the improvement activities seemed to have little effect, there was no blood collection error during the campaign. We will continue to carry out ABO blood type registration of inpatients in Severance Hospital and we should try to be a safe blood transfusion.

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TRANSFUSION REACTIONS AND COSTS OF LEUKOCYTE-DEPLETED VS LEUKOCYTE-POOR PACKED RED CELLS FOR THALASSEMIA PATIENTS AT KING CHULALONGKORN MEMORIAL HOSPITAL

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Background: Thalassemia is the most common inherited blood disorder in Thailand. Regular transfusion remains the main therapy for thalassemia major. To reduce adverse reactions, leucoreduced blood components are recommended. Currently, leukocyte-depleted pack red cells (LDPRC) and leukocyte-poor red cells (LPRC) are available, but there has been no study comparing these 2 products.
Aims: To compare adverse reactions between leukocyte-depleted pack red cells (LDPRC) and leukocyte-poor red cells (LPRC)
Methods: This is a retrospective study of all transfusion reactions in chronically-transfused thalassemia patients from 2011 to 2013. Data were obtained from medical records. Costs of treatments were also estimated.
Results: In total, 388 thalassemia patients were transfused with 13,616 red cell units and 36 adverse transfusion reactions (Urticaria 23 events, Febrile non-haemolytic transfusion reaction (FNHTR) 9 events, other 4 events) were observed in 8,441 transfusions (0.4%). Transfused red cell components comprised LDPRC 8,372 units (61.5%) of pre-storage, 361 units (2.6%) of post-storage LDPRC, 4,827 units (35.5%) of LPRC and 56 units (0.4%) of pack red cell (PRC). Most paediatric patients were transfused with LDPRC (88.6%). Comparing with adult patients, were transfused with LDPRC (33.2%) and LPRC (66.2%). 25 transfusion reactions occurred in 4,998 (0.5%) in paediatric compare with 9 transfusion reactions occurred in 3,443 (0.3%) in adult ($P = 0.053$). In adult patients, 5 transfusion reactions occurred in 1,016 (0.49%) Pre-storage LDPRC transfusions and 4 in 2,210 (0.18%) LPRC transfusions ($P = 0.12$). For 3 Febrile Non Hemolytic Transfusion Reactions (FNHTR) in adult, 2 events occurred in 1,016 LDPRC transfusion and 1 event in LPRC transfusion ($P = 0.23$). In adults, the costs of pre-storage LDPRC and LPRC per unit were 2,260 baht and 1,139 baht, respectively.
Summary/Conclusions: Transfusion reaction rates in LDPRC and LPRC groups were not different. However, transfusion reactions may be underreported and other factors may interfere with the results.

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Abstract has been withdrawn.

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APPLICATION OF NEW BLOOD TRANSFUSION PROTOCOLS

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Background: Blood transfusions are linked to risks and complications such as transfusion reactions, immunomodulation and Transfusion Related Lung Injury (TRALI). Furthermore, there is a growing evidence about the high number of critical patients transfused that still has not tangible benefits from such treatment. To ensure the proper allocation of blood products has been reworked the good use of blood manual that specifies guidelines for proper transfusion, blood requesting module, and the proper administration of informed consent
Aims: The purpose of the study is to show the data, in the year 2016, concerning the application of new protocols at the transfusion service Aversa
Methods: We examined 350 requests for transfusion medicine advice, received at the transfusion service Aversa
The following parameters were examined:

- Presence or absence of certain diagnosis
- Presence or absence of martial profile (serum iron, ferritin, transferrin)
- Presence or absence of adequate medical history performed by the staff of the transfusion service about the main signs of blood loss (melena, rectal bleeding, epistaxis, metrorrhagia, menorrhagia, chronic inflammatory diseases)

Results: The results showed that of the 350 requests for transfusion advice there are 40 inappropriate requests. Of these, 26 due to iron deficiency (missing martial profile at the time of the EC request), 8 due to gynecological problems (access to the emergency room for breakthrough bleeding or menorrhagia), 5 due to problems gastroenterological / intern (esophageal varices, gastric ulcers, hemorrhoids), 1 due to disease otolaryngologist (a rendu Osler syndrome)
Summary/Conclusions: The correct clinical practice based on medical history, clinical signs and laboratory findings have enabled the correct application of the new protocol thus avoiding unnecessary transfusions 40, with benefits not only for the patient but also for the entire health care system

P-710

INHS (IRANIAN NATIONAL HAEMOVIGILANCE SYSTEM) ACHIEVEMENTS FROM JANUARY 2009 TO DECEMBER 2016: A MODEL FOR MIDDLE EAST AND SOUTH ASIA COUNTRIES

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Background: Haemovigilance is a set of surveillance procedures covering the whole transfusion chain from the collection of blood components to the follow-up of its recipients, intended to collect and assess information on unexpected or undesirable effects resulting from the therapeutic use of blood products, and to prevent their occurrence and reoccurrence. Haemovigilance system is a mandatory system in Iran that is supported by the law and since 2009 this system was established in some hospitals as a pilot project, and then in 2012, its implementation became mandatory in all hospitals. It should be noted that donor related reactions are collecting in donor vigilance system and it is not included here.
Aims: Defining the frequency and incidence of ATR in IRAN.
Methods: Trained physicians on Haemovigilance were responsible for diagnosis of ATR and filling in the manual form for every case identified. All hospitals have to use the same *blood transfusion adverse reaction report form* which has been prepared by central Haemovigilance office of IBTO. The records were periodically sent in the first step to one of the 31 blood transfusion centres in the country and, after primary checking and in the second step to the central Haemovigilance office of IBTO for further evaluation.
After reviewing, reports were not consistent with the definition were excluded and the rest of the information was entered in software.
Since the blood components consumption was not available, therefore, the incidence of adverse effects was calculated based on the number of units of blood products distributed in hospitals.
All information entered into the software SPSS version 16. Data analysis was performed with the chi- square test.
Results: A total of 12,039 of transfusion reactions were reported. Most complications were reported from Tehran Province
The incidence of ATR was equivalent to 1.1 reactions every 1,000 units of blood components distributed. The majority of reported reactions were acute or immediate.
Summary/Conclusions: From January 2009 to December 2016 this system was established in 688 (75.6%) hospitals (from 910 hospitals across the country). The incidence of ATR was equivalent to 1.1 reactions every 1,000 units of blood components distributed.

Alternatives to blood transfusion

P-711

IMPLICATIONS OF FERRIC CARBOXYMALTOSE ADMINISTRATION IN THE IMMEDIATE POSTOPERATIVE PERIOD OF PATIENTS UNDERGOING CARDIAC SURGERY

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Background: In the immediate postoperative period, cardiac surgery patients usually present with iron deficiency postoperative anemia. Treatment of such anemia should consist of restoring iron, folic acid and B12 deposits, avoiding the transfusion of packed red blood cells (RBC). Within the essential points for the control of perioperative transfusion NICE recommends the use of oral and intravenous iron before and after surgery in patients with anemia due to iron deficiency. After surgery there is an alteration in the iron absorption capacity at the intestinal level, which would advise its IV administration.
Aims: To assess the efficacy of cardiac post-surgery administration of Ferric Carboxymaltose (FC) in the immediate postoperative period by the measurement of hemoglobin at hospital discharge.
Methods: We have conducted a retrospective observational study including patients over 16 years of age undergoing programmed cardiac surgery. Exclusion Criteria: Age <16 years, urgent cardiac surgery, ventricular assist devices (ECMO), cardiac transplantation, previous haematological disease, and transfusion rejection. We analyzed 483 patients, 294 consecutive patients from January to July 2013 (Preprotocol group) whose results were compared with 189 patients from October 2015 to January 2016 (Postprotocol group) who started treatment with postoperative FC after Ferric metabolism parameters compatible with iron deficiency anemia were detected. The variables analyzed were retrospectively extracted, for data processing and statistical studies a statistical program was used.
Results: The overall transfusion rate in the Pre-protocol group was 90.1% and in the Post-protocol 60.3% ($P < 0.001$). Preoperative hemoglobin (Hb) was analyzed in both preprotocol (PreP) and postprotocol groups (PostP), being 13.18 g/dl (SD:±1.86) and 13.12 (SD:±1.88) respectively ($P = 0.75$). The observed transfusion threshold was not different in both groups (PreP Hb = 7.70, SD = ±1.17 and PostP Hb = 7.65, SD = ±0.64, $P = 0.68$). The minimum Hb in CPB in the preP group was 7.48 g/dl (SD:±1.33) vs 8.31 (SD:±1.13) in the post-P group ($P < 0.001$). In the postoperative period in the post-P group, FC was administered in 64.6% of the patients. The Hb at the critical care unit discharge was in the preP group of 9.77 g/dl (SD:±1.7) and 9.44 (SD:±1.15) in postP ($P < 0.001$). At hospital discharge, the Hb in the preP group was 10.47 g/dl (SD:±1.24) and in the postP was 10.33 (SD:±1.31); $P = 0.24$. When we analyzed only the post-protocol group and compared the treated and not treated with FF we found that at the hospital discharge the group treated with FC had an Hb level of 10.56 g/dl (SD:±1.35) and in the untreated group of 10.04 g/dl (SD:±1.19); $P = 0.012$.
Summary/Conclusions: The preoperative Hb level is not improved probably due to protocol non-compliance. The use of pre surgery restrictive fluid therapy, the reduction of priming, etc. have been deployed correctly. Since the PostP group received fewer transfusions and presented no different Hb levels, we could infer that the recovery of Hb is due in part to IV Fe, which would have an effect after critical care discharge. Within the postP group, those treated with FC are discharged with significantly higher Hb levels.

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Abstract has been withdrawn.

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FIBRINOGEN USE GUIDED BY ROTATIONAL THROMBOELASTOMETRY (ROTEM) AS PART OF A BLOOD SAVING PROTOCOL IN CARDIAC SURGERY

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Background: Perioperative bleeding in cardiac surgery results in a high blood component consumption, especially in those procedures that require extracorporeal circulation. Rotational thromboelastometry has been used to control bleeding and in that way may reduce blood transfusion.
Aims: To analyze the use of fibrinogen once rotational thromboelastometry has been introduced as part of the hemostasis monitorization in the blood saving protocol in patients undergoing Cardiac Surgery
Methods: We conducted a retrospective observational study including patients over 16 years of age undergoing programmed cardiac surgery. Exclusion Criteria: Age <16 years, urgent cardiac surgery, ventricular assist devices (ECMO), cardiac transplantation, previous haematological disease and transfusion rejection. We analyzed 482 patients, 294 consecutive patients from January to July 2013 (Preprotocol group) whose results were compared with 189 patients studied from October 2015 to January 2016 (Postprotocol group). The preoperative patients history was reviewed to obtain the demographic, clinical and laboratory variables and, subsequently, we collected the intraoperative and postoperative variables of their clinical evolution. The variables analyzed were retrospectively extracted, for data processing and statistical studies, an statistical program was used
Results: Both groups of patients did not differ in terms of demographic and clinical characteristics (age, sex, logistic Euroscore and Euroscore II) ($P > 0.05$). Preoperative fibrinogen level was not statistically different in both groups. In the preprotocol group, ROTEM was used in 28% of patients, while in the postprotocol group it was used in 93.1% of patients ($P < 0.001$). 50.4% patients who were monitored by ROTEM received fibrinogen while those who were not, empirically received fibrinogen in a 3.2% proportion ($P < 0.001$). Patients who received fibrinogen had a mean MFCFIBTEM value of 9.87 mm (SD: ± 4.2) and those who did not receive fibrinogen 15.47 mm (SD: ± 5.3). The overall transfusion rate in the Pre-protocol group was 90.1% and in the Post-protocol 60.3% ($P < 0.001$). Regarding red cell transfusional rate, it was 82.1% in patients not monitored with Rotem, while in those with ROTEM it was 64.7% ($P < 0.001$). In contrast, both plasma and platelet use were unaffected by the use of ROTEM (absence of statistically significant differences). On the other hand, there was no difference in the rate of reintervention due to hemorrhage (9.1% in the global group), of both periods (preprotocol and postprotocol).
Summary/Conclusions: The use of ROTEM seems to influence in the overall and red cell transfusion rate decrease, but probably due to the increased use of fibrinogen while maintaining the same use of "hemostatic" blood components, ie, platelets and plasma. Therefore, we could infer that this diagnostic test is useful to anticipate in the control of hemostasis with agents that help to control coagulation reducing blood loss and therefore the use of red cell concentrates.

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Abstract has been withdrawn.

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IMPLEMENTATION OF A BLOOD SAVING PROTOCOL IN THE CARDIAC SURGERY AREA AND ITS TRANSLATION IN THE TRANSFUSION RATE

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Background: Perioperative bleeding in cardiac surgery results in a high blood component consumption, especially in those procedures that require extracorporeal circulation. Various scientific societies have proposed clinical blood saving guidelines, following a multidisciplinary and multimodal approach with recommendations

ranging from the use of drugs to intraoperative strategies or postoperative management. Following these guidelines we have elaborated and implemented a "BLOOD SAVING PROTOCOL IN CARDIAC SURGERY SETTING" in our hospital.
Aims: Assessment of the implementation efficacy of a blood saving protocol in patients undergoing cardiac surgery according to the transfusion rate variation
Methods: We conducted a retrospective observational study including patients over 16 years of age undergoing programmed cardiac surgery. Exclusion Criteria: Age <16 years, urgent cardiac surgery, ventricular assist devices (ECMO), cardiac transplantation, previous haematological disease and transfusion rejection. We analyzed 482 patients, 294 consecutive patients from January to July 2013 (Preprotocol group) whose results were compared with 189 patients studied from October 2015 to January 2016 (Postprotocol group). The preoperative patients history was reviewed to obtain the demographic, clinical and laboratory variables and, subsequently, we collected the intraoperative and postoperative variables of their clinical evolution. The variables analyzed were retrospectively extracted, for data processing and statistical studies, an statistical program was used
Results: Both groups of patients did not differ in terms of demographic and clinical characteristics (age, sex, logistic Euroscore and Euroscore II) ($P > 0.05$). The overall transfusion rate in the Pre-protocol group was 90.1% and in the Post-protocol 60.3% ($P < 0.001$). If we analyzed red blood cell concentrates, the transfusion rate in the Preprotocol group was 84.6% and in the Postprotocol 54.5% ($P < 0.001$). These differences were also observed for plasma and platelets transfusion rate (36.2% vs 20.6%, $P < 0.001$ and 40.6% vs 29.1%; $P = 0.01$ respectively). Analysis of the fibrinogen use showed higher rates in the postprotocol group (16.5% vs 47.1%, $P < 0.001$). Aortic valve replacement surgery in the preprotocol period (49 patients) had a transfusion rate of 93.9% and in the postprotocol (51 patients) 52.9% ($P < 0.001$). Coronary bypass in the preprotocol period (96 patients) had a transfusion rate of 87.5% and in the postprotocol (51 patients) 47.1% ($P < 0.001$).
Summary/Conclusions: Overall, red cells, plasma and platelets transfusion rates, have been decreased and fibrinogen consumption has increased after the introduction of the protocol. The decrease in the overall and red cells transfusion rates have also been observed in certain surgery subgroups (aortic valve replacements and cardiac by-pass). We must continue to work to ensure the implementation of all the measures specified in the protocol in order to continue with the transfusion rate reduction improvement.

P-716

IATROGENIC ANAEMIA, HOW THE TRANSFUSION PRACTITIONER CAN INFLUENCE

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Background: Iatrogenic anaemia is a condition in which a low haemoglobin concentration and haematocrit result from large or frequent venepuncture to patients for laboratory tests. It is also known as Hospital Acquired Anaemia.
The Patient Blood Management programme within the UK identified the minimisation of blood sampling from patients to reduce the risk of iatrogenic anaemia as a key objective. It has also been identified by the American Association of Blood banks (AABB) in their Choosing Wisely campaign and the Australian Patient Blood Management guidelines.
In the 2013 within the UK a Patient Blood Management Survey to hospitals demonstrated that only 21% of Hospital Trusts had a policy to minimise the volume and frequency of blood sampling to minimise iatrogenic anaemia. This number rose to 40% in the 2015 survey.
Aims: Transfusion Practitioners (TP) are key to driving the patient blood management (PBM) message within their clinical environments, and although some key areas within PBM may feel beyond the scope of control or influence of the TP, undertaking an iatrogenic anaemia project can deliver some positive changes that benefit patients, with an added benefit of possible financial gains due to reduction in both blood sampling and reduction in blood usage.
Methods: This talk will review how the TP can undertake an audit and collect data to understand the number of tests taken and the impact on patient's haemoglobin, how to engage with stakeholders in highlighting the benefits of this quality improvement project, consider what other work has previously been done on iatrogenic anaemia and discuss some potential strategies to reduce the volume of blood removed.
Results: By demonstrating the positive impact this can have, it adds momentum to the overall PBM programme within the hospital setting.
Summary/Conclusions: The TP role is not just about making big changes that alter clinical transfusion practice, but can be about making small positive incremental gains that contribute to the overall PBM picture.

P-717

Abstract has been withdrawn.

Cellular therapies

Stem cell and tissue banking, incl. Cord blood

P-718

Abstract has been withdrawn.

P-719

MESENCHYMAL STROMAL CELLS OVEREXPRESSING NGAL AMELIORATE ACUTE KIDNEY INJURY IN RAT MODEL

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Background: Acute kidney injury (AKI) is one of the most common health- threatening diseases in the world. There is still no effective medical treatment for AKI. Recently, Mesenchymal stromal cell (MSC)-based therapy has been proposed for treatment of AKI. However, the microenvironment of damaged kidney tissue is not in favorable for survival of MSCs which would be used for therapeutic intervention.
Aims: It was hypothesized that MSCs overexpressing NGAL/Lcn2 would enhance cell survival after transplantation and restore kidney function
Methods: In this study, we genetically manipulated MSCs to up-regulate lipocalin-2 (Lcn2) and investigated whether the engineered MSCs (MSC-Lcn2) could improve cisplatin-induced AKI in a rat model.
Results: Our results revealed that up-regulation of Lcn2 in MSCs efficiently enhanced renal function. The MSC- Lcn2 up-regulates expression of HGF, IGF, FGF and VEGF growth factors. In addition, they reduced molecular biomarkers of kidney injury such as KIM-1 and Cystatin C, while increased the markers of proximal tubular epithelium such as Aqp-1 and CK18 following cisplatin-induced AKI.
Summary/Conclusions: Overall, here we over-expressed Lcn2, a well-known cytoprotective factor against acute ischemic renal 37 injury, in MSCs. This not only potentiated beneficial roles of MSCs for cell therapy purposes, but also suggested a new modality for treatment of AKI.

P-720

HUMAN PLATELET LYSATE MODIFICATIONS AFFECT MESENCHYMAL STEM CELL BIOLOGY DEPENDING ON CELL ORIGIN

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Background: Cell-based therapeutics demand xeno-free culture conditions. Due to abundant growth factors, pooled human platelet lysate (pHPL) has been shown to be a valuable human alternative for fetal bovine serum (FBS). However, there are many different protocols described for the production of pHPL. This lack of standardization hampers comparability of *in vitro* studies and clinical trials testing mesenchymal stem/progenitor cell (MSPC) therapy.
Aims: Aim of this study was to compare three different pHPL-preparation methods for manufacturing of MSPCs isolated from umbilical cord (UC), white adipose tissue (WAT) and bone marrow (BM). Based on preliminary published reports, we hypothesized that pHPL modifications equally or better support MSPC propagation compared to FBS while maintaining phenotype, trilineage potential and stemness.
Methods: pHPL preparations were tested for biochemical parameters and a panel of growth factors (ProcartaPlex multiplex immunoassay). UC-, WAT- and BM-MSPCs (each n = 3) were isolated and cultured using four different medium types: Standard-medium (pHPL), fibrinogen depleted pHPLS-medium (serum-converted pHPL), mechanically fibrinogen depleted medium and FBS-medium. Immunophenotyping and differentiation assays, as well as proliferation and colony forming unit (CFU) assays were conducted for all MSPCs over four passages at low cell density. qRT-PCR to analyze the stemness factors KLF4, SOX2, cMYC and OCT4 was performed.
Results: Biochemical parameters were comparable in all pHPL preparations. Although distinct growth factors were significantly decreased in fibrinogen-depleted pHPL, all pHPL-based culture media significantly enhanced MSPC propagation compared to FBS. While WAT-MSPCs could be cultured efficiently in all pHPL-based media, UC- and BM-MSPCs showed significantly decreased proliferation in fibrinogen-depleted pHPL media. MSPCs showed a stable phenotype (CD73+/90+/105+ and CD14−/19−/34−/45−/HLA-DR-), and osteogenic, adipogenic and chondrogenic differentiation potential independent of MSPC source or medium composition. Depending on primary cell source, expression of KLF4, SOX2 and cMYC was significantly elevated in pHPL- compared to FBS-derived MSPCs.
Summary/Conclusions: Despite some variations with regard to tissue source, MSPCs showed significantly enhanced proliferation in modified pHPL- compared to FBS-medium, while maintaining trilineage differentiation potential. Our results indicate that pHPL-modifications support the proliferative and clonogenic capacities, reflecting a more undifferentiated status of MSPCs.

P-721

Abstract has been withdrawn.

P-722

IMPACT OF NEUROPEPTIDE Y (NPY) ON *EX VIVO* EXPANSION OF UMBILICAL CORD BLOOD HEMATOPOIETIC STEM CELLS

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Background: Umbilical cord blood (UCB) has been recognized as a valuable source for hematopoietic stem cell transplantation due to its availability, easy collection and the lower risk of GVHD. However, the number of hematopoietic stem cells (HSCs) in each unit of UCB is still a limitation for clinical application; *ex vivo* expansion of HSCs to obtain a sufficient number for therapeutic purposes is necessary.
The sympathetic nervous system (SNS) and neurotransmitters regulate HSCs self-renewality, proliferation and differentiation in the bone marrow. However the specific role of NPY as one of the leading neuropeptide of SNS remains unknown.
Aims: The aim of this study was to evaluate the proliferative effect of NPY on the CB-HSCs in *ex vivo* culture.
Methods: Three units of UCB were selected for evaluation. CD34+ cells were isolated from total nucleated cells (TNC) of each UCB with the MACS magnetic absorption column. The purity of CD34+ cells were verified with flow cytometry. *Ex vivo* culture of CB-HSCs were performed in two conditions: one with cytokines (SCF, FLT3 L, TPO) as a control group and the other with 1.0 micro molar NPY treatment in addition to cytokines. TNC number and CD34+ cell number were calculated on day 7. Colony formation assay were performed in methylcellulose medium and incubated in 5% CO2 for 14 days. The student *T*-test was used for comparison among various groups in SPSS version 23.0.
Results: *Ex vivo* expansion of CB-HSCs after 7 days resulted in significant increase of CD34+ hematopoietic stem cells of NPY-treated groups in comparison to control group. Hematopoietic colonies including CFU-E, CFU-GM and CFU-GEMM scored on day 14. We observed different cell colonies identified as Erythrocyte, Granulocyte and Mix. NPY-treated CD34+ hematopoietic cells after 7 days of culture retained their ability to differentiate into blood cells.
Summary/Conclusions: Our study demonstrated that Neuropeptide Y can successfully supported expansion of CD34+ hematopoietic stem cells with retaining their potential of differentiate into various cell lineage after 7 day culture period with cytokine supplementation.

P-723

ESTIMATION OF THE OPTIMAL CORD BLOOD INVENTORY SIZE FOR PROVISION OF A COST-EFFECTIVE TERRITORY-WIDE PUBLIC CORD BLOOD BANKING SERVICE FOR HONG KONG WHEN CONSIDERING 8 HLA LOCI INCLUDING HLA-C

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Background: Hong Kong Red Cross Catherine Chow Cord Blood Bank (CBB) has previously determined the optimal inventory size of 10,000 umbilical cord blood (UCB) units aiming at achieving a high probability of finding at least one matched UCB unit (matching ≥4 out of 6 Class I and II loci, i.e. HLA-A, -B and –DRB1) for local Chinese patients on a cost-effectiveness basis. Recent international guidelines recommended extending HLA-matching of UCB for haematopoietic stem cell transplantation to 8 HLA loci including HLA-C.
Aims: This study attempted to determine the optimal inventory size of our CBB when considering 8 HLA loci including HLA-C for provision of HLA-matched UCB units.
Methods: We estimated the probabilities of finding 1 or more compatible UCB units for 936 previous local HLA-typed patients (192 children & 744 adults) in that requests for search had been initiated as a function of seeking four levels of HLA matching (5, 6, 7 and 8 out of 8 loci by HLA-A, -B, -C and -DRB1 low resolution HLA typing) according to various UCB inventory levels, which were constituted by simulation through random drawing of HLA typing results from the database of Hong Kong Bone Marrow Donor Registry.
Results: With inventory levels of 5,000, 10,000, 15,000 and 20,000 UCB units, 25.1% (95% CI = ±2.8%), 32.9 ± 3.0%, 34.7 ± 3.1% and 40.4 ± 3.1% of patients respectively will have at least 1 unit available that is 8 out of 8 loci matched (median: 1, 2, 2 and 2 donors per patient respectively); 63.0 ± 3.1%, 73.6 ± 2.8%, 76.9 ± 2.7% and 81.0 ± 2.5% respectively will have at least 1 unit available that is 7 out of 8 loci matched (median: 4, 6, 7 and 10 donors per patient respectively); 91.6 ± 1.8%, 95.8 ± 1.3%, 96.9 ± 1.1% and 97.6 ± 1.0% respectively will have at least 1 unit available that is 6 out of 8 loci matched (median: 12, 24, 36 and 52 donors per patient respectively); 98.3 ± 0.8%, 99.6 ± 0.4%, 99.6 ± 0.4% and 99.7 ± 0.4% respectively will have at least 1 unit available that is 5 out of 8 loci matched (median: 54, 111, 162 and 236 donors per patient respectively). At an inventory level of 10,000 and above, there is no significant difference between the probabilities of finding at least one unit of HLA-matched UCB at 5 out of 6 (HLA-A, -B and -DRB1) and 6 out of 8 (including HLA-C) loci matching level ($P > 0.1$).
Summary/Conclusions: Ethnic Chinese accounts for 95% of the local population. Our findings showed that the same inventory level of 10,000 high-quality UCB units is considered optimal for Hong Kong, even when HLA matching is extended from 6 to 8 loci including HLA-C. Doubling the inventory level to 20,000 UCB units will yield ≥1 compatible UCB for additional 0.1% and 1.8% of patients at 5 and 6 out of 8 allele-matched level respectively; the increase in chance of finding suitable units at these matching levels for transplantation follows the law of diminishing return and is considered as marginal in the context of cost-effectiveness.

P-724

COMPARISON OF QUALITY BETWEEN LONG-TERM STORAGE AND SHORT-TERM STORAGE CORD BLOOD FOR IMPROVEMENT OF STORAGE POLICY IN KOREAN PUBLIC CORD BLOOD BANK

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Background: Cord blood (CB) has an advantage for easier access than those of bone marrow or peripheral blood stem cells because CB is a ready-made product. Improvement of haploidentical transplantation resulted in decreased usage of CB, therefore public cord blood banks had a financial difficulty. The cost of storage for CB has been increased cumulatively. There was no regulation for storage duration of CB in Korean public banks.
Aims: We have evaluated the quality of long-term storage and short-term storage of CB to establish storage policy.
Methods: We used 34 units of CB cryopreserved for less than 1 year and 31 units of CB cryopreserved for up to 14 years in Busan Gyeongnam Public Cord Blood Bank. Total nucleated cell count (TNC), CD34+ cell count, colony-forming units (CFUs)-GM, and cell viability by Eosin-Y staining were examined for quality evaluation. Post-thawing data between long-term storage CB and short-term storage CB were compared by Student *t*-test.
Results: Cell viability of long-term and short-term storage CB was 90.9 ± 4.5% (mean ± standard deviation) and 88.6 ± 2.7%, respectively (P = 0.01). The TNC and CD34+ cell count were not significantly different between long-term and short-term storage CB, respectively (5.5 ± 1.4 vs 5.1 ± 0.9 ($\times 10^8$) for TNC and 45.2 ± 21.3 vs 45.6 ± 26.7 (uL) for CD34+). CFU-GM of short-term storage CB was significantly higher than that of long-term storage CB (63.6 ± 26.1 vs 49.9 ± 22.7 CFU-GM (0.95 mm^2), P = 0.02)
Summary/Conclusions: It was difficult to conclude that the quality of long-term storage CB was inferior to short-term storage CB. New CB storage policy based on the pattern of cord blood usage would be needed.

P-725

CORD BLOOD BANKING ACTIVITY IN THESSALONIKI CORD BLOOD BANK (TSCB) GREECE: 8 YEARS DATA ANALYSIS

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Background: Public TSCB established in 2009 and accepts cord blood units (CBUs) for allogeneic transplantation. Housed in the G. Papanicolaou Hospital, Department of Hematology-BMT Unit, National Health System. Umbilical cord blood (UCB) represents a stem cell source for transplantation. In principle, transplant graft content on CD34+ progenitors and especially colony-forming units (CFUs) correlate with the speed of engraftment. Cell-derived microparticles (MPs) are small membrane vesicles which have been detected in cord blood. MPs are considered as markers of cell activation, as well as apoptosis
Aims: The aim of this study is to analyze certain parameters of UCBs as well as MPs of CD34+ cells in cord blood units and to investigate their potential role to the cord blood quality.
Methods: Volume reduction and RBC depletion is performed by the automated system SEPAX (Biosafe). Controlled-rate freezers are used after 10% DMSO addition and transplants are stored in liquid nitrogen tanks. Labeling system follows NETCORD–FACT standards. Evaluation of total nucleated cell number (TNC) pre-and post-processing and CD34+ cell number and viability, viral and microbiological screening is performed. Molecular intermediate resolution HLA typing (A, B, C and DRB1) analyzed in EFI accredited laboratory. Nucleated RBC, ABO typing, post-thawing CD34+ cell number and viability analysis is performed. For assessment of CFUs, pro- post-processing and post-thawing samples assays were performed using commercially prepared complete methylcellulose medium., Methocult GF H4434 (Stem Cell Technologies, CA). Cells plated in duplicate and store 14 days at 37°C in humidified air and 5% CO_2, granulocyte-macrophage (CFU-GM), erythroid (BFUE) and multipotential (CFU-GEMM) colonies were scored by microscopic examination. The MPs were isolated after centrifugation of the plasma and determined after incubation with Annexin V and CD34 by flow cytometry.
Results: TSCB is a member of Bone Marrow Donors Worldwide, the Hellenic Transplant Organization and the Netcord Foundation (associate). By the end of 2016, TSCB processed and stored 5,291 units. Mean volume of collected CBUs was 87.9 ml. The post-processing number of CD34+ cells was 3.3 × 10^6/unit with viability 91%. Univariate analysis using Spearman's correlation showed that the pro-processing CD34+ number is significantly positive correlated to the post-processing (P = 0.00, rho = 0.918) and post-thawing CD34+ number (P = 0.002, rho = 0.665). The mean TNC number was 77.9 × 10^7/unit with recovery 82.4%. The recovery of CFUs pro-processing vs post-thawing was 94.5%. CD34+ number is significant correlated with the CFUs. On univariate analysis found that the number of annexin V and CD34 positive microparticles (AnnV+/CD34 + MPs) (pre-processing vs post-processing) has statistically significant mean positive correlation (P < 0.000, rho = 0.681) whereas the number of AnnV+/CD34 + MPs post-thawing was not statistically significant. Post-processing AnnV+/CD34 + MPs has moderate significant correlation with the absolute number of pro-processing CD34+ cell per unit (P < 0.016, rho = 0.443), post-processing (P < 0.035, rho = 0.393) and post-thawing CD34+ cells per unit (P < 0.049, rho = 0.457). The number of AnnV+/CD34+ MPs (pro-processing and post-processing) showed moderate correlation with pro-BFUe and pro-CFU-GM (P = 0.006, rho = 0.500, P = 0.011, rho = 0.463, P = 0.005, rho = 0.504 and P = 0.025, rho = 0.415 respectively).
Summary/Conclusions: The number of CD34+ cells and AnnV+/CD34+ MPs can be valuable parameters to evaluate the CBU quality.

P-726

Abstract has been withdrawn.

P-727

VALIDATION OF STERILITY CONTROL ON CORD BLOOD BASED ON THE MICROBIAL CONTROL OF CELLULAR PRODUCTS AS DESCRIBED IN THE EUROPEAN PHARMACOPOEIA (2.6.27)

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Background: The Foundation for Accreditation of Cellular Therapy requires a validated method for Cord Blood (CB) sterility testing. Until now the sterility control was performed by the blood institution. Because we want to outsource the sterility test, a validation was needed. Here we describe the results of the method validation in accordance with the European Pharmacopoeia 7.0, 2.6.27 as recommended by the Superior Health Council.
Aims: To verify that microorganisms can be detected in the presence of the product to be tested by the method used.
Methods: During processing of a Cord Blood unit (CBU) a mixture of 10 ml plasma and 10 ml red blood cells (RBCs) was inoculated into BacT/ALERT FA plus and FN plus bottles (Biomérieux, France). After inoculation of the mixture the bottles were spiked with 10–100 CFU of one of the following microorganisms: A. brasiliensis, P. acnes, S. aureus, B. fragilis, S. pyogenes, P. aeruginosa, C. albicans, B. subtilis, C. sporogenes and Y. enterocolitica. Each organism was tested with plasma/RBCs mixture from 3 different CBUs.
For logistic reasons we have assessed the influence of a delayed entry of inoculated bottles. All tests were done both with inoculation and entry within 2 h as well as with inoculation and an entry delay of 18–24 h. Inoculated bottles were stored at room temperature before entry.
The bottles were sent to the microbiology laboratory where they were incubated for maximum 10 days at +-37°C. The laboratory recorded the time of loading and the time the bottle became positive or the time the bottle was unloaded. Positive bottles were investigated with gram stain, subculture (Blood agar and thioglycolate broth) and identification (MALDI-TOF MS).
The test was considered successful if the microorganism seeded was detected in the BacT/ALERT FA and/or the FN bottle within 10 days.
Results: Growth of all microorganisms (except A. brasiliensis and P. acnes) was detected within 48 h. Growth of P. acnes was detected after 6–8 days of incubation.
Only 2 out of 3 bottles spiked with A. brasiliensis were flagged positive (repeated tests). The gram stain and the subculture of the positive bottle remained negative. To resolve this problem an additional Sabouraud slanted tube was inoculated with 2 ml

of the plasma/RBC mixture and spiked with 10–100 CFU of A. brasiliensis. Growth of A. brasiliensis was detected after 4 days in all the tubes tested.
For bottles with a delayed entry the detection time was comparable with the detection time of the bottles with no delay entry or was extended with a maximum of 24 h.
Summary/Conclusions: The results confirm that by using the BacT/ALERT FA plus and FN plus bottles, growth of all microorganisms, with exception of A. brasiliensis, could be detected in the presence of the plasma/RBC mixture by incubating the bottles at +-37°C for maximum 10 days.
Detection of A. brasiliensis in the plasma/RBC mixture can be obtained by additional inoculation of a Sabouraud agar.
Delayed entry of the bottles extended the detection time with maximum 24 h.

P-728

Abstract has been withdrawn.

P-729

ALGORITHM OF CRYOPRESERVATION PROTOCOL FOR STEM CELLS IN BLOOD TRANSFUSION INSTITUTE OF VOJVODINA

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Background: Cryopreservation protocol for stem cells from peripheral blood was introduced into the regular procedure in the Stem cells bank of Blood Transfusion Institute of Vojvodina in January 2015. Implementing this procedure enabled that complete stem cell transplantation procedures are performed in the same place (the Department of Hematology in Clinical Center of Vojvodina in Novi Sad and Blood Transfusion Institute of Vojvodina also in Novi Sad), unlike the previous decade when the Department of Hematology performed procedures in cooperation with the Military Medical Academy in Belgrade.
Aims: The aim is to present algorithm of cryopreservation protocol for autologous stem cells from peripheral blood which is used at our Institute.
Methods: Separation of autologous stem cell can be made within hematologic patients who satisfy medical criteria for stem cells transplantation. Modification of protocol for cryopreservation of stem cells from peripheral blood by Clinical hospital center in Zagreb – Clinical institute for transfusion medicine and transplantation biology Rebro, according to our equipment and materials, we formed our own cryopreservation algorithm. Patient is previously prepared with so called "mobilization" treatment at the Clinic of Hematology followed by collection of stem cells performed with the cell separator. Collected stem cells are deplasmated and mixture of cryoprotectants 99.99% solution of DMSO, and 5% albumin in a ratio of 5: 1: 4 is added. The initial freezing of stem cells is performed by the Bavarian protocol (56 min at 100°C). Storing and keeping the cells are at a temperature of −188°C in vapors of nitrogen liquid till the moment of reinfusion of the patient.
Results: From January 2015 until March 2017 cryopreservation procedures of autologous stem cells were performed in 20 patients: 16 males and 4 females. Frequency of diagnosis within patients: 12 patients diagnosed with Myeloma multiplex, 4 non Hodgkin Lymphoma, 2 patients with Burkitt lymphoma and 1 patient with myeloma and 1 with Hodgkin's Lymphoma. Reinfusion of stem cells was carried out in 7 patients who have had an adequate therapeutic response in accordance with the controlled number of CD34 in the frozen control samples. Two patients died during treatment before reinfusion.
Summary/Conclusions: The achieved results of the treatment fully justify the implementation of a modified method of cryopreservation, its validity and are achieving optimal cost effect.

P-730

Abstract has been withdrawn.

P-731

ERYTHROID CULTURE BASED ON DIFFERENTIATION OF PROGENITOR CELLS FROM LEFTOVER BUFFY COATS PRODUCED IN THE REVEOS AUTOMATED BLOOD PROCESSING SYSTEM

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Background: Efforts to culture red blood cells (RBCs) for transfusion purposes are ongoing in numerous research laboratories worldwide. In addition, smaller-scale erythroid culture is one of many approaches toward better understanding of erythropoiesis, enucleation and RBC biology. Our center recently changed from traditional blood component separation to the Reveos(R) automated blood-processing system. Most buffy coats (BCs) after whole-blood donations were previously used for platelet production but leftover BCs are now discarded. BCs are still popular among scientists, mainly as a source of peripheral leukocytes but we asked the question if these leftover bags could be useful for erythroid culture.
Aims: We aimed to set up and evaluate a protocol for *in vitro* erythroid culture of $CD34^+$ progenitor cells extracted from Reveos(R) BCs.
Methods: Total CD34+ cells in Reveos(R) BCs were measured using flow cytometry and the BD Cell Enumeration Kit. Following pre-enrichment and fractionation on a density gradient, the mononuclear cells were labeled with anti-CD34 and separated by magnetic particles using an EasySep™ magnet. Culture conditions for the extracted $CD34^+$ cells were divided into phases I-III, each lasting for 7 days. Phase I is an expansion phase, phase II an erythroid differentiation phase and phase III a terminal differentiation phase. StemSpan™ SFEM II culture medium containing bovine serum albumin, human insulin and iron-saturated human transferrin was used in all phases. In phase I, SFEM II was supplemented with SCF, IL-3, FLT3L, TPO and dexamethasone, in phase II with SCF, IL-3, EPO and dexamethasone and in phase III with 30% serum, EPO and holo-transferrin. Fetal bovine serum (FBS) or human serum (HS) were used initially in parallel cultures, to compare their influence on proliferation rate and terminal differentiation. In all cultures, the total number of cells and cell viability were determined by cell counting in a Bürker chamber after trypan blue dye staining. The cultures were carefully monitored and diluted to 0.4×10^6/ml when entering phase II. Erythroid differentiation was analyzed on day 19, 21 and 23 by flow cytometry with anti-GPA-APC, anti-Band3-PE and anti-CD49d-PE-Cy7. The frequency of erythroblasts and enucleated cells present in the cultures were counted on May-Grünwald-Giemsa stained cytospin samples.
Results: We found the $CD34^+$ cell content by flow cytometry in BCs (n = 11, volume of 9.5–13.5 ml) to be $0.2–1.5 \times 10^6$ with a mean of 93% viability. Thereafter progenitor cells were isolated and subjected to a 3-phase erythroid culture procedure to evaluate their potential to expand and differentiate. Cells cultured in 30% HS in phase III were less viable but showed slightly higher co-expression of CD49d and Band 3 compared to cultures grown in 30% FBS (86% vs 75% on day 19, and 95% vs 86% on day 21), as well as a higher proportion Band 3-positive, CD49d-negative cells, indicating an increased proportion of late erythroblasts and reticulocytes. In order to promote end-stage maturation, HS cultures were favored. After 23 days in culture with HS in phase III, 8–21% of the cells were enucleated and the cultures contained ~60% erythroblasts.
Summary/Conclusions: Extraction of $CD34^+$ hematopoietic progenitors at acceptable viability from leftover Reveos(R) BCs is feasible and convenient. Despite their small volume and the processing cycle, these BCs provide suitable starting material for small-scale, experimental erythroid cultures directly from single donors. Conditions in this culture model can be fine-tuned further to focus on different aspects of erythropoiesis.

P-732

PENTAISOMALTOSE MAY ACT AS AN ALTERNATIVE TO DMSO. DATA FROM AN ENGRAFTMENT STUDY WITH CRYOPRESERVED HUMAN HEMATOPOIETIC PROGENITOR CELLS (HPCS) IN IMMUNODEFICIENT NSG MICE

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Background: Hematopoietic stem cell transplantation often involves cryopreservation of stem cell products. The standard cryoprotective agent (CPA) currently used is dimethyl sulfoxide (DMSO). However, DMSO show concentration-related toxicity and can cause side effects when administered to patients. Therefore, more and more research aims to meet the increasing demand for non-toxic cryopreservation alternatives devoid of DMSO- and xeno-additives.

Aims: The aim of the study was to compared the engraftment of HPCs cryopreserved in Pentaisomaltose or DMSO in a preclinical humanized immunodeficient NSG mouse model

Methods: Human hematopoietic progenitor cells (HPCs) from mobilized peripheral blood were cryopreserved in either DMSO- or a Pentaisomaltose-based cryoprotective medium, using a controlled rate freezer. Outcome measures were 1) post-thaw recovery of $CD34^+$ cells and clonogenic potential assessed by colony forming cell assay (CFC) and 2) engraftment in NSG mice.

Results: *In vitro* recovery and CFC data were found to be comparable for Pentaisomaltose and DMSO. *In vivo* data from the NSG mouse model did not show significant difference in human $CD45^+$ levels in peripheral blood 8 weeks post transplantation, or in bone marrow 16 weeks post transplantation. The frequency of $CD34^+CD38^{low/negative}$ cells and the lineage distribution of myeloid and lymphoid cells in the BM were also comparable.

Summary/Conclusions: The present data indicates that mobilized HPCs cryopreserved in Pentaisomaltose- or DMSO-based freeze media, maintain comparable engraftment potential in the NSG mouse model.

Collection, processing, storage and release

P-733

MULTI CENTRE AUDIT OF PERIPHERAL BLOOD STEM CELL (PBSC) COLLECTIONS PERFORMED BY THE THERAPEUTIC APHERESIS UNITS OF NHS BLOOD AND TRANSPLANT

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Background: In order to improve quality and reduce variation in practice between the NHSBT therapeutic apheresis service units, we undertake an ongoing audit of PBSC collections from all five of our adult units based around England. This is essential for performance evaluation and comprises a JACIE requirement for accreditation.

Aims: To determine the efficiency of PBSC collections and the effectiveness of the regimens used for mobilization.

Methods: Prospective data from 777 consecutive donors covering the period from 1-1-2016 to 31-12-2016 were analysed for the following parameters: pre CD34 count, CD34 yield per procedure, total CD34 dose collected per patient, CD34 collection targets requested by clinical teams. Donors with failed mobilisation who did not undergo apheresis were excluded from the analysis. The efficiency of PBSC procedures was determined by calculating the collection efficiency (CE) and the correlation coefficient between pre CD34 count and yield per procedure.

Results: From the 777 donors, 574 were autologous (74%) and 203 were allogeneic (26%).

The 574 autologous donors underwent in total 857 procedures. The median CD34 target dose for these donors was 4×10^6/kg. 299 autologous donors (52%) achieved the target dose with 1 procedure, 194 (34%) with 2 procedures and 55 (10%) with 3 procedures. The median pre CD34 count was 28/µl while 30 of autologous donors underwent a collection with CD34 count <10 µl. The median CD34 yield per procedure was 2.61×10^6/kg and the median of total CD34 dose collected per autologous donor was 5.29×10^6/kg. 2% of autologous donors collected a total CD34 dose $<1 \times 10^6$/kg and 7% between $1–2 \times 10^6$/kg.

The 203 allogeneic donors underwent in total 285 procedures. The median CD34 target dose for these donors was 5×10^6/kg. 114 allogeneic donors (56%) achieved the target dose with 1 procedure, 75 (37%) with 2 procedures and 7 (3%) with 3 procedures. The median pre CD34 count was 47/µl while 4 allogeneic donors underwent a collection with CD34 count <10 µl. The median CD34 yield per procedure was 3.69×10^6/kg and the median of total CD34 dose collected per donor was 6.52×10^6/kg. 2% of allogeneic donors collected a total CD34 dose $<1 \times 10^6$/kg and 3% between $1–2 \times 10^6$/kg.

The median CE for autologous donors was 54% while 67% of all procedures had a CE >40%. The median CE for allogeneic donors was 47% while only 39% of all procedures had a CE >40%. The median correlation coefficient between pre CD34 count and CD34 yield per procedure was $r^2 = 0.86$ for the autologous and $r^2 = 0.34$ for the allogeneic collections.

Summary/Conclusions: The majority of autologous and allogeneic donors achieved the target CD34 dose with one procedure. 91% of autologous and 95% allogeneic donors collected a transplantable CD34 dose of $>2 \times 10^6$/kg.

The low CE and poor correlation coefficient seen in the allogeneic donors confirm previous findings that have shown that the efficiency of procedures in allogeneic donors is lower than in autologous donors.

The CE of autologous procedures for each units was >50%. Based on this evidence the CE of 50% can be used in the future in a formula that will predict for each individual donor the blood volume required to be processed to achieve the target CD34 dose.

P-734

COMPARISON OF TWO APHERESIS PROTOCOLS FOR PERIPHERAL BLOOD STEM CELLS COLLECTION WITH SPECTRA OPTIA

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Background: The Spectra Optia (Terumo BCT) is an apheresis system developed based on COBE Spectra platform, which has been used in our department for collection of peripheral blood stem cells (PBSC) in patients for autologous use, since 2013. Two protocols for collection of PBSC) have been developed by Terumo BCT: Mononuclear Cell System (MNC), considered a discontinuous-flow cell collection, and the continuous mononuclear cell collection (CMNC)

Aims: A retrospective review of 57 collections of PBSC performed using Spectra Optia in 33 adult patients eligible for mobilized autologous stem cell apheresis, using two different protocols (MNC e CMNC) and compare performances between them

Methods: We review data from 57 collections of HPSC in 33 patients: 24 collections with the MNC protocol and 33 collections with the CMNC protocol. The parameters analysed of the patients were: sex, underlying diagnose, weight, blood volume, white blood cells (WBC), platelets (PLT) and blood CD34+ cells pre-apheresis. The parameters analysed of the collection product were: WBC, CD34+ cells yield, procedure run time, volume, and collection efficiency (CE2%)

Results: Demographics characteristics of the patients were similar in both groups.

The blood volume processed was 22% higher on CMNC protocol than in MNC protocol (9,431 vs 7,716 ml, respectively), though the running time per procedure was 5% lower (168 min vs 177 min, respectively). The product volume was 45% higher on CMNC protocol (158 ml vs 109 ml, respectively).

The collection efficiency (CE2%) is similar with both protocols (MNC: 63.1% and CMNC: 64.8%, respectively). However, product CD34+/kg collected by CMNC protocol is 27% higher compared to MNC protocol (3.46 vs 2.72×10^6/kg, respectively),

despite the blood CD34+ cells pre-apheresis was comparable (39.9 vs 35.6/μl, respectively)

Summary/Conclusions: Both protocols are effective for the collection of PBSC, with very high collection efficiency. CMNC protocol is easier to use when compared to MNC protocol, although more dependent on operator expertise. However, this potential disadvantage allows optimization of collection parameters, improving product quality, as evidenced by CD34+ cells yield

P-735

EFFECT OF COLLECTION DEVICE ON PRODUCT QUALITY AND DONOR SAFETY IN ALLOGENEIC AND AUTOLOGOUS TRANSPLANTATION WITH CD34+ HPCS

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Background: In hematopoietic transplantation G-CSF mobilized peripheral blood stem cells collected by apheresis have been used as a common standard. Apheresis technology has been refined introducing a new cell separator with an automatic electronic interface management.

Aims: The data of allogeneic and autologous apheresis products collected with either device are analysed with regard to product quality and donor safety. Allogeneic grafts without, and autologous HSC concentrates after cryopreservation were included.

Methods: 125 peripheral blood stem collections were performed in 116 individuals. In 44 related donors, 47 stem cell harvests were done, 20 applying the manual COBE Spectra cell separator (V5.0; MNC programme, Terumo BCT) (CS), 27 the automatic Spectra Optia (V11.0; cMNC programme, Terumo BCT) (SO). 78 apheresis products from 72 patients for autologous stem cell rescue after high dose chemotherapy – 35 of which being collected on CS, the remaining 43 on SO – were cryopreserved with 10% DMSO in a controlled rate freezer (516.16, Planer) and stored in the gas phase above liquid nitrogen. Stem cell concentrates were analysed on a blood counter (KX-21, Sysmex) and a flow cytometer (Calibur, BD) for differential count, vital NCs and CD34+ HPCs and in allogeneic donations for lymphocyte subpopulations. Aliquots of cryopreserved products were thawed and analysed for total NCs, MNCs, viability and clonogenic potential in cytokine-supplemented semi-solid media (Methocult H8444, Stem Cell Technologies).

Results: In allogeneic collections the yields in procedures with CS vs CO averaged: vital TNCs 10.4 *10e10 vs 10.1*10e10, monocytes 2.0*10e10 vs 2.6*10e10, lymphocytes 2.6*10e10 vs 3.2*10e10, CD34+ HPCs 6.2*10e8 vs 7.5*10e8, platelets 71.0*10e10 vs 43.3*10e10, respectively. In autologous apheresis products cell contents were in the mean TNCs 7.1*10e10 vs 6.5*10e10, monocytes 1.5*10e10 vs 1.8*10e10, lymphocytes 2.6*10e10 vs 0.7*10e10, CD34+ HPCs 9.2*10e8 vs 7.6*10e8, platelets 17.4*10e10 vs 7.3*10e10, respectively. These mostly significant differences were in contrast to the findings in cryopreserved products: with either cell separator, stem cell harvests of 12.2*10e6 CD34+/kg BW vs 11.8*10e6 CD34+/kg BW were frozen in 2.7 bags with a total volume of 348 ml (CS) and 333 ml (SO), respectively. In thawed aliquots NC viability was 95.8% (CS = SO) corresponding to CFU-GM doses of 23.6*10e5/kg BW vs 18.1*10e5/kg BW, respectively. Ratios of CFU-GM and CD34+ HPC doses were 17.8% (CS) and 15.9% (SO), respectively.

Summary/Conclusions: These findings demonstrate a higher enrichment of MNCs in the stem cell collections performed with the automatic SO as compared to the former manual CS which was in parallel to a significantly lower trapping of platelets. This appears to be favourable for donor safety. But the quality of cryopreserved autologous HPC harvests obtained with either device was similar.

P-736

NEW TECHNIQUE FOR PBPC COLLECTIONS USING OPTIA SPECTRA, CMNC

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Background: Autologous and allogeneic transplantations of peripheral blood progenitor cells (PBPC) are considered as a routine therapy in patients with hemato-oncological diseases. PBPC collections are performed by leukapheresis technique in the standard or large volume leukapheresis (LVL) regimen. Recently, Cobe Spectra, ...Cobe" separators were replaced by the next generation Spectra Optia, ...Optia" (both Terumo), and new system requires finding of the optimum collection technique.

Aims: After an introduction of Optia, v. 11, CMNC we tried to find the optimum collection regimen in order to prepare at least the same quality PBPC grafts as by Cobe. The results of 107 PBPC collections which were performed by Cobe (44) and Optia (63) in 107 patients with malignant lymphomas were evaluated. Forty four of patients were subsequently transplanted with autologous grafts, and the time of engraftment in the number of neutrophil leukocytes and platelets was estimated.

Methods: Autologous PBPC were collected by Cobe and Optia in patients with the precollection concentration of CD 34+ cells in blood higher than 20 v μl. Collections were performed by LVL with processing more than three total blood volumes (TBV) of the patients. The results from the first procedures were analyzed. The target dose of CD34+ cells for transplantation higher than 5×10^6/kg recipient was required. PBPC were transplanted to 44 patients after administration of high dose chemotherapy. The results are expressed as medians and their ranges.

Results: The patient's precollection concentration of CD 34+ cells in Cobe group was 73 (21–629) in μl, and in Optia 84 (22–716) in μl. Volume of processed blood was in Cobe 3.5 (3–4.5)×TBV, which corresponded with 18 (11–20) l of blood, while in Optia 3.5 (3–4.9) × TBV were processed, which corresponded with 17.7 (10–20.8) l of blood.

The number of CD 34+ cells prepared from one collection by Cobe was 8.7 (2.4–86) $\times 10^6$/kg, while by Optia 10.9 (2–61) $\times 10^6$/kg. The differences were not statistically significant (Mann Whitney, a = 0.05). The products from both types of separators contained comparable numbers of platelets: Cobe 1.8 (0.8–6.3) $\times 10^{11}$ and Optia 1.6 (0.6–8.2)$\times 10^{11}$, but the percentage of platelets collected from blood volume to the bag was in Cobe 56.5% (34.5–86) while in Optia 46% (18.7–121). The differences were significant (Mann-Whitney, a = 0.05). In the number of 44 transplanted patients time of engraftment in the number of neutrophil leucocytes (higher than 0.5 $\times 10^9$/l) was in Cobe 11 (8–12) days, and in Optia 11 (8–13) days. Time of engraftment in the number of platelets (higher than 50 $\times 10^9$/l) corresponded in Cobe with 13 (10–35) days while in Optia 15 (9–123) days.

Summary/Conclusions: Cobe Spectra and Spectra Optia are efficient systems in the process of PBPC collections, and enabled to prepare the sufficient numbers of CD 34+ cells for transplantation from one LVL procedure. No serious adverse reactions in the course of collections have been observed.

P-737

PERIPHERAL BLOOD STEM CELL (PBSC) COLLECTION IN AN INFANT WEIGHING 6.2 KG: A CASE REPORT

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Background: While PBSC collection has become a safe procedure for adults, only a few reports exist about its efficacy, safety and feasibility in paediatric patients, especially extremely low-weight infants. Performing a PBSC harvest procedure using apheresis technology in a very low body weight infant (<10 kg) can be very challenging. Special attention is needed with reference to total blood volume, vascular access, product volume, anticoagulant ratio and circulatory balance.

Aims: We report successful PBSC collection and transplantation in a 6 months old infant with 6.2 kg body weight and suffering from stage IV neuroblastoma.

Methods: Harvest of PBSC was started after mobilisation with high-dose chemotherapy and G-CSF, as soon as the peripheral blood CD34+ cell count was above 20/cumm. Collections were performed using a COM.TEC cell separator primed with gamma irradiated, white cell-depleted and phenotype matched packed red cells diluted with saline to match the patient's haematocrit. Triple lumen femoral line was used for vascular access and the blood flow was maintained at 10 ml/min.
Results: 1,700 and 1,240 ml which is equivalent to 3.4 and 2.4 times the patients total blood volume respectively was processed to obtain PBSC product volume of 50 and 31 ml on Day 1 and Day 2 of the harvest. 4.54 $\times$ 10^6/kg body weight of CD 34 positive cell dose was collected cumulatively. The product was cryopreserved using 8.7% DMSO in 1:1 proportion. The stem cells were infused after conditioning regime, on Day 9 after the harvest. The patient had DMSO toxicity, which was managed promptly. The baby transplanted successfully with myeloid engraftment on Day + 11 and platelet engraftment on Day +14. Transfusion support of packed RBC's (200 ml) and Platelets (200 ml) was needed during the engraftment period. The patient was discharged on Day +21 and is doing well on current follow up at Day +90 post-PBSC infusion.
Summary/Conclusions: PBSC harvesting using continuous flow cell separators is safe and effective, even in low-weight infants of 6.2 kg body weight. Taking into account that the side effects related to the procedure were mild and well tolerated, we conclude that even a large volume leukapheresis is safe in very small infants. This is the first case reported in our country to the best of our knowledge.

P-738

ASSOCIATION OF CD34+ CELL DOSE WITH HEMATOPOIETIC RECOVERY AFTER AUTOLOGOUS STEM CELL TRANSPLANTATION

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Background: Hematopoietic recovery after autologous stem cell transplantation (ASCT) is associated with the number of reinfused CD34+ stem cells. For successful engraftment 0.75 $\times$ 10^6/kg CD34+ cells required.
Aims: To evaluate the impact of reinfused CD34+ cells dose on hematopoietic recovery in patients with oncohematological and autoimmune diseases after ASCT.
Methods: 169 cases of ASCT in patients with autoimmune (n = 87) and hematological (n = 82) disease were studied. Bleeding was not registered, cases of deaths – 2. Stem cell mobilization was performed with granulocyte-colony stimulating factors. In 106 patients was mobilized $\geq$2 $\times$ 10^6/kg CD34+ cells, in 63 patients $\leq$2 $\times$ 10^6/kg CD34+ cells.
Results: Hemoglobin recovery >8 g/dl is 4 days earlier in patients with autoimmune disease who received $\geq$2 $\times$ 10^6/kg CD34+ cells than in recipients of lower dose (9.9 and 14 day). Association between duration of anemia and dose of transplant in patients with hematological disease was not revealed. Reinfusion of lower dose of transplant among oncohematological patients associated with increased needs for red blood cells transfusions – these patients require $\geq$3 units of RBC 2 times more. Risk of thrombocytopenia and platelet transfusions is lower in patients with autoimmune disease who received $\geq$2 $\times$ 10^6/kg CD34+ cells. It is established that dose of stem cells do not affect the duration of thrombocytopenia <10 and <20 $\times$ 10^9/l and mean needs for donor platelets. Characteristics of neutropenia after ASCT are not associated with the dose of reinfused stem cells in patients with autoimmune diseases with. However the negative correlation (−0.6 and −0.8) between transplant and time of neutrophil recovery >0.5 and >1.0 $\times$ 10^9/l was revealed. Reinfusion of higher dose in compare with lower dose of cells in oncohematological patients associated with shorter duration of neutropenia (7.6 and 9 days) and faster neutrophil recovery (10 and 11.6 day).
Summary/Conclusions: Duration of thrombocytopenia and platelet recovery time >10 and >20 $\times$ 10^9/l, duration of neutropenia and time of neutrophil recovery >0.5 and >1.0 $\times$ 10^9/l, also needs for platelet transfusions are lower in oncohematological patients who received $\geq$2 $\times$ 10^6/kg CD34+ cells. Risk of anemia and red blood cells transfusions, time of hemoglobin recovery, risk of thrombocytopenia <10 $\times$ 10^9/l and platelet transfusions is lower in patients with autoimmune diseases received $\geq$2 $\times$ 10^6/kg CD34+ cells. Duration of thrombocytopenia and neutropenia is not associated with the dose of transplant.

P-739

EVALUATION OF FACTORS THAT INFLUENCE COLLECTION EFFICIENCY OF HEMATOPOIETIC STEM CELLS FROM PERIPHERAL BLOOD

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Background: Successful collection of mononuclear cells (MNC) and CD34+ peripheral blood cells is prerequisite for successful transplantation of haematopoietic stem cells (HSC).
Aims: The aim of our study was to investigate possible predictive factors that could influence hematopoietic stem cell yield.
Methods: The investigation group is concluded of 120 autologous and allogeneic donors undergoing apheresis collections of HSC in the Institute for Transfusion Medicine of RM from 2008 till 2016. The influence of possible predictive factors (demographic characteristics, laboratory parameters, collection characteristics in both groups, and mobilization strategy and the disease characteristics in autologous donors) on the MNC and CD34+ cells collection efficiency was determined by multiple regression analysis.
Results: There were 226 apheresis collections, 182 in 90 autologous and 44 in 30 allogeneic donors, mean 2 apheresis (range 1–3) in autologous and 1.5 apheresis (range 1–2) in allogeneic donors. Collection efficiency of MNC in autologous donors was significantly influenced by preapheresis platelet count (P = 0.045576), mobilization strategy (P = 0.044071), type of set for apheresis collection (P = 0.042254), number of apheresis cycles in one procedure (P = 0.037069) and number of cycles of chemotherapy (P = 0.033519). Collection efficiency of CD34+ cells in autologous donors was affected by number of apheresis cycles in one procedure (P = 0.014608). Statistical relationship was not found, between the investigated predictive variables of interest and collected number of MNC and CD34+ cells in allogeneic donors, by multiple regression analysis.
Summary/Conclusions: Development of universal guidelines for initiation of apheresis procedure based on preapheresis blood count values is needed for optimization of collection efficiency of hematopoietic stem cells.

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Abstract has been withdrawn.

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Abstract has been withdrawn.

P-743

THE EFFECT OF ALTERED TEMPERATURE ON CONCENTRATIONS OF GROWTH FACTORS AND CYTOKINES IN PLATELET-RICH PLASMA

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Background: Platelet-rich plasma (PRP) is an autologous product derived from patient's own whole blood by utilizing the process of gradient density centrifugation. Multiple studies have demonstrated a role for PRP in accelerating and facilitating improved response to injury. The medical potential of PRP is largely due to the growth factors and cytokines derived from platelets. Recent reports disclosed the influence of the cycling freeze-thaw activation on growth factor and cytokine

concentrations, but no study focus on middle-window of temperature effect, ranging from 4°C to 37°C.
Aims: The purpose of the study is to standardize the preparation of PRP with activated platelets in different temperature setting, which does not require the addition of any substrates and thus not alter the contents of the sample. We analyzed the effect of different temperature settings on the quantification of various growth factors and cytokines.
Methods: PRP samples were collected from 20 healthy donors and a half volume underwent incubation for platelets activation. EGF, VEGF, PDGF-AB and interleukin (IL)-8, tumor necrosis factor (TNF)α and anti-inflammatory IL-1RA, IL-10 were assayed in 2 days after incubation in different temperature settings. There are two patterns of settings, including consistent pattern incubated in low-temperature (4°C, 15°C), mild-temperature (22°C, control), high temperature (37°C) for 60 min and alternative pattern with cooling-warming cycles (4–37°C) for 60 min (2 cycles, 4 cycles, 6 cycles). The resulting growth factor and cytokine concentrations from PRP of different temperature settings were analyzed and compared using enzyme-linked immunosorbent assays.
Results: The growth factors (EGF, VEGF, PDGF-AB) levels were moderately increased in low-temperature (4°C, 15°C) incubation compared to the control group (22°C). Pro-inflammatory cytokines interleukin (IL)-8, tumor necrosis factor (TNF)α and anti-inflammatory IL-1RA, IL-10 was also increased with low temperature, that depends on donors' health status. There was mildly increase in EGF, VEGF, PDGF-AB concentrations of groups of alternative pattern, especially in higher cooling-warming cycles.
Summary/Conclusions: This method is useful for *in vitro* laboratory experiments because it uses a physical, not chemical, mechanism of platelet activation. Despite wide variation of concentration of platelets-derived growth factors and cytokines, alternative cooling-warming system is still the cost-effective and convenient method for PRP preparation.

Clinical applications

P-744

Abstract has been withdrawn.

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P-748

PLATELET INTERACTION WITH NANO- AND MICROFIBROUS MATERIALS FOR THE TISSUE ENGINEERING

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Background: Tissue engineering is an interdisciplinary field that applies the principles of chemistry, physics, material science, engineering, cell biology and medicine to the development of biological substitutes that restore, maintain or improve tissue/organ functions. Nano- and microfibrous materials are essential components for a range of applications in the fields of tissue engineering and regenerative medicine since their morphology resembles significant features of native extracellular matrix. Fibrous scaffolds have a high surface to volume ratio which promotes cell adhesion, migration, proliferation and differentiation. As polymeric fibrous scaffold fabrication techniques strive to create structures that mimics the structure and function of ECM, the need for increased scaffold bioactivity becomes more pronounced. Platelet-rich plasma (PRP) contain over 300 bioactive molecules. Activated PRP has been shown to contain platelet-derived growth factor (PDGF), transforming growth factor-β (TGF-β), vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), epidermal growth factor (EGF) and others in elevated concentrations. PRP has the potential to deliver a combination of GF and cytokines capable of stimulating cellular activity. Incorporation of platelets into scaffold will help to promote scaffold integration within native tissues and increase the overall patency of fibrous structure
Aims: We used PRP to study interaction between native platelets and fibrous materials with the different morphologies (porosity, fibres diameter – micro, nano).
Methods: PRP was prepared as whole blood-derived platelet concentrate (7×10^6 PLT/ml). Fibrous materials were made from polycaprolactone by electrospinning, centrifugal spinning and met-blown methods. Prepared and sterilized materials were incubated for 2 h with PRP. The degree of platelet adhesion was measured using MTT method. Interaction of PLT with materials was evaluated by fluorescence microscopy (FITC-anti CD41) and electron microscopy (SEM). The GFs release was monitored by SDS PAGE.
Results: PLT adhere on both – nano and micro fibrous materials. The adhesion rate of PLT was significantly higher in materials containing nanofibres – either electrospun or in combination with meltblown. SEM pictures shows, that PLT on nanofibres are spread on surface (across more fibres), whereas on microfibrous material they are attached on single fibres. We observed also differences in the aggregation behaviour of platelets on materials with various fibre diameters – PLT form aggregates rather on microfibres than nanofibres. GFs release from PLT is significantly faster when materials contain nanofibres.
Summary/Conclusions: Initial results of our study show that the presence of microfibers in the scaffold structure has an influence on the activation of platelets and leads to a more gradual release of GFs.
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Abstract has been withdrawn.

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MOBILIZED HEMATOPOIETIC STEM CELLS FROM PERIPHERAL BLOOD AS A PREFERRED SOURCE IN ALLOGENEIC STEM CELL TRANSPLANTATION IN ADULT PATIENTS WITH HEMATOLOGIC MALIGNANCIES

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Background: Mobilized hematopoietic peripheral blood stem cell (PBSC) has been widely used for allogeneic transplantation in different hematologic malignancies. Optimal donor and recipient outcomes require maximized stem cell collection efficiency.
Aims: The aim of our study is to present our experience of 16 years in collecting of PBSC in healthy donors for allogeneic stem cell transplantation.
Methods: This is a retrospective study performed in the Institute for Transfusion Medicine of Republic of Macedonia and University Hematology Hospital for period from 2001 till December 2017. All donors were HLA typed and matched; they were fully informed on the donation procedure and signed an informed consent for donation. Minimum dose required to ensure successful and sustained engraftment was 2×10^6/kg CD34+ cells and 2×10^8/kg mono-nucleated cells (MNC). PBSC harvesting was performed with continuous flow cell separator Baxter C53000 and COBE Spectra using conventional-volume apheresis processing the 2–2.5 total blood volumes per apheresis. A femoral catheter was used for harvesting and Acid Citrate Dextrose formula A is used for anticoagulation. Recombinant human granulocyte colony-stimulating factor (G-CSF) is used to mobilize PBPC for collection. Harvesting of PBSC is usually performed after 4 to 5 days of G-CSF subcutaneous administration at a dose of 10 μg/kg body weight.
Results: All the donors were siblings of the patients treated at the University Hematology Hospital. There were 144 apheresis procedures performed in 89 healthy sibling donors. There were 56 males and 33 females, aged 19–55. The single procedure usually took 3–4 h and the volume of collected stem cells was 50–220 ml. The needed number of MNC and CD34+ cells was successfully collected by 1.6 apheresis (range 1–2). There were 10 ABO incompatible donors. Procedures for mobilization

and collection of PBPC from healthy donors are generally well tolerated. The main adverse effects of the apheresis procedure were bone pain as reaction of G-CSF and numbness of the extremities as reaction of ACD-A (hypocalcemia), which occur rarely and were very mild. The collected PBSC were used in allogeneic stem cell transplantation in patients with: acute myeloid leukemia – 53.9%, acute lymphoblastic leukemia – 17.9, chronic myeloid leukemia – 8.0%, severe aplastic anemia – 4.5%, myeloproliferative disorders – 4.5%, myelofibrosis – 4.5%, non-Hodgkin lymphoma – 3.4%, chronic lymphoblastic leukemia – 1.1%, Hodgkin disease – 1.1% and multiple myeloma – 1.1%.

Summary/Conclusions: The needed number of MNC and/or CD34+ cells was successfully collected by 1.6 apheresis, and successful allogeneic transplantation of PBSC was enabled.

P-751

THE THERAPEUTIC EFFECT OF ACTIVATED PLATELET-RICH PLASMA (APRP) ON HUMAN UMBILICAL CORD BLOOD MESENCHYMAL STEM CELLS IN EAE MODEL

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Background: Current clinical research supports the immunomodulatory and neuroprotective effects of administered Mesenchymal Stem Cells (MSCs) in Multiple Sclerosis.

Aims: The therapeutic efficacy of expanded hUCB-MSCs in aPRP was evaluated in EAE model in comparison with ordinary cultured hUCB-MSCs .

Methods: hUCB-MSCs were collected from the umbilical cord blood. MSCs were cultured in DMEM-low glucose with 10% FBS or 10% aPRP. After inducing EAE in mice, the hUCB-MSCs were transplanted and disease progress was evaluated. Then, the mice were sacrificed, and histological analysis of CNS tissue were done.

Results: The comparison of weight and disease severity between groups showed that there is significant difference between experimental group which received cultured hUCB-MSCs in medium containing aPRP and other groups ($P < 0.05$).

Summary/Conclusions: Our findings demonstrated that aPRP supplemented medium not only improves the proliferation but also maintains functional activity of UCB-MSCs and it can be a suitable substitution for FBS in clinical purposes.

Clinical immunogenetics

HLA in transfusion medicine

P-752

HLA AND KIR LIGAND REPERTORY IN AML PATIENTS IN AN INDIAN COHORT

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Background: The observation by Lilly et al that increased or decreased susceptibility of mice to virus-induced leukemia was linked to H-2^k and H-2^b respectively prompted Amiel et al to search for associations between histocompatibility antigens and pathologic conditions in humans. He reported a significant association for HLA B5 in patients with Hodgkin's disease. Since then numerous studies investigated HLA associations with many other diseases and leukemias.

Aims: The present retrospective study was initiated to analyze the association of HLA A, B, C, DRB1, DQB1 alleles and haplotype among AML patients and controls in Indian population. Later HLA association according to morphological, cytogenetic, hematological, and clinical classifications was analyzed along with the KIR ligand association in prognosis as it may have an effect in outcomes post- Bone Marrow Transplant.

Methods: A total of 453 patients with AML and 2630 healthy donors which included the sibling and parents referred to our HLA and Immunogenetics laboratory were selected for the study. The allele frequency of HLA A, B, C, DRB1, DQB1 alleles and of the HLA-A, B, DR haplotype was compared between patients and controls to understand the susceptibility and protection. Later HLA association was correlated on the basis of morphological, cytogenetic, hematological, and clinical classifications in AML patients. The odds ratio (OR) and 95% confidence intervals limits (CI) (SPSS version 20.0) was calculated and values with $P < 0.05$ was considered to be significant.

Results: Among the AML patients, overall frequencies of HLA-C*14, DQB1*05 and DQB1*06 alleles were significantly increased (OR 5.83, 1.81 and 1.65, respectively. Among females also HLA-C*14, DQB1*05 showed statistically significant increased frequencies with odds ratios of 31.52 and 3.22 respectively. Therefore, in Indian cohort among females HLA- C*14 and DQB1 may be a risk factor for AML. On the other hand DRB1*04($P = 0.36$, OR0.58) may be a protective allele among females, albeit not significant. Among the adults (age≥15 years) the frequency of DQB1*05 was significantly increased and DRB1*11 showed decrement (OR 1.7, 0.064), respectively. In pediatric age group (age < 15 years) HLA-C*04, *15 and DQB1*06 showed significantly higher frequencies (OR 5.23, 3.46 and 4 respectively). We noticed that among females the HLA C1 KIR ligand whereas, in the pediatric group HLA C2 KIR ligand was associated with risk of AML.

Summary/Conclusions: This study highlights the importance of HLA and non- HLA genes, their association, role in prognosis of disease in different age groups and genders, to improvise therapeutic outcomes for Indian population.

P-753

STUDY ON HLA-DRB1*15:03, HLA-DRB1:09 AND HLA-DRB1*11 TO DETERMINE PREDISPOSING ALLELES OF ALLOANTIBODY PRODUCTION IN IRANIAN PATIENTS WITH THALASSEMIA

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Background: Repetitive Red Blood Cell transfusion is the mainstay of therapy in thalassemia patients. The major complication of transfusion therapy is Red Blood Cell Alloimmunization. The genetic markers of recipients are still poor recognized. This is the first study in Iran to survey the association of HLA-II alleles and alloantibody production in thalassemia patients.

Aims: The present study was investigate to determine the relationship between HLA-DRB1*15:03, HLA-DRB1*11 and HLA-DRB1*09:01 alleles and alloimmunization in Iranian Thalassemia patients.

Methods: Antibody screening tests were performed by tube method and HLA-DRB1 genotyping was determined by SSP-PCR in 59 alloimmunized and 205 non-alloimmunized patients. HLA-DRB1 allele's frequencies were compared between alloantibody positive and negative groups by chi-squared test.

Results: HLA-DRB1*15:03 allele frequency was significantly different between groups ($P = 0.000$, $OR = 4.193$). There was correlation between HLA-DRB1*11 and anti-K ($P = 0.000$, $OR = 6.643$). There was not any association between HLA-DRB1*09:01 and alloimmunization ($P = 0.350$).

Summary/Conclusions: According to our results, detecting HLA-DRB1*15:03 and HLA-DRB1*11 alleles as a genetic markers in the pre-transfusion test could determine responder patients and improve transfusion safety.

P-754

Abstract has been withdrawn.

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Abstract has been withdrawn.

P-756

INVESTIGATION OF SENSITIZATION AND SPECIFICITY OF HLA ANTIBODIES AMONG PATIENTS ON TRANSPLANT WAITING LIST

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Background: Human leukocyte antigens (HLA) antibody has been proven to cause immune-mediated disease like transplantation rejection and transfusion related acute lung injury (TRAL1). The antibody screening for the donors was an essential diagnosis indicator before transplantation and monitor tool after transplantation. The specificities of HLA antibodies of patients on the transplant waiting list were considered to correlate with HLA frequency.
Aims: The aim of the study is to investigate the positive rate and specificity of HLA antibodies and observe the HLA frequency in Taiwan.
Methods: All the blood of 1,149 patients was collected from 2015 to the end of 2016. The screening of those sera samples and identification of antibody specificity were carried out using the Luminex system and MATCHIT Antibody software. According to the geography, patients were classified into three parts (northern, central and southern).
Results: From the HLA antibody screening results, the positive rate of the patients in class I, class II and both class I and II showed 21.06%, 24.02% and 12.53%, respectively. The presence of HLA-A, B and C antibodies in class I were 37.60% (91/242), 38.84% (94/242) and 25.21% (61/242) and HLA-DQ, DR in class II were 28.26% (78/276), 34.42% (95/276), respectively. High frequency of HLA class I, class II and both class I and II antibody in patients from northern, central and southern showed the same level. Some HLA antibodies expressed higher frequency, A2 (9.09%), A24 and A6602 (7.44%), B7 (4.96%), Cw1 and Cw7 (4.55%), DQ7 (16.3%), DR1 (22.83%), respectively.
Summary/Conclusions: High frequencies of HLA antibodies among patients in Taiwan need to be further quantified thus stratifies the risk of immunization, which could help clinician to assess the selection of donors graft for sensitized patients.

P-756-a

CLINICAL RELEVANCE OF PRE-TRANSPLANT BLOOD TRANSFUSIONS IN KIDNEY TRANSPLANT PATIENTS

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Background: Alloantibodies against human leukocyte antigens (HLA) represent an important immunological barrier to successful organ transplantation. HLA alloimmunization is caused by exposure to various sensitizing factors such as blood transfusions, pregnancy and/or organ transplantation. Of the three, the main potentially modifiable factor are blood transfusions.
Aims: The aim of this study is to assess the frequency of exposure to pre-transplant blood transfusions and evaluate their effect on HLA alloimmunization in kidney transplant recipients using two different HLA antibody screening techniques: complement-dependent cytotoxicity (CDC) and Luminex.
Methods: This is a retrospective study that included HLA antibody screening results of kidney recipients transplanted in Clinical Hospital Centre Rijeka from March 2012 until the end of December 2015. The screening of HLA antibodies has been performed periodically every three months, four times per year by CDC and more sensitive Luminex assays in parallel. Information on blood transfusions was obtained from patients, their nephrologists and medical records.
Results: Of total 118 kidney recipients, pre-transplant transfusions received 59 (50%) patients, 37 (31.36%) male and 22 (18.64%) female. 35 patients (29.66%) received at least one red blood cell (RBC) unit. To the combination of previous transfusions and pregnancies 17 (14.41%) women were exposed, transfusions and transplants five (4.24%) recipients and two (1.69%) patients experienced all three sensitizing events. Almost all transfused patients have received RBC concentrates (with or without leukocyte reduction) before registration on the waiting list. While being on the waiting list only four (3.39%) patients were transfused, one for the first time. All of them have received leukoreduced blood products. Control group consisted of 32 patients who were not exposed to any known sensitizing event.
Pre-transplant sera of 40 (67.80%) transfused patients were negative. HLA antibodies were detected in 7 (11.86%) patients by CDC and Luminex (CDC+LUM+) and in additional 12 (20.34%) by Luminex only (CDC-LUM+). According to the technique, the difference between the number of patients with HLA antibodies detected by CDC and Luminex technique is not statistically significant. However, the risk of developing HLA antibodies after exposure to blood transfusions is statistically significant when combined with other sensitizing events (OR: 3.325; 95% CI 1.020–10.837; $P = 0.046$). Also, the allograft and patient survival in this group of kidney transplant recipients was significantly lower compared with control group ($P = 0.0237$).
Summary/Conclusions: Pre-transplant blood transfusions are the most frequent sensitizing factor in patients on kidney waiting list. In combination with previous pregnancy and/or transplantation, transfusions represent significant immunogenic stimulus. In relation to CDC, Luminex is a more sensitive and specific technique in HLA antibody detection, revealing higher number of sensitizing patients, although the difference is not statistically significant. While patients on the waiting list in Clinical Hospital Centre Rijeka were minimally exposed to blood transfusions, almost all transfused patients received RBC concentrates before becoming a registered transplant candidates. Due to detrimental effect of HLA immunization on graft survival and transplant outcome, avoidance of transfusions whenever possible still remains the key strategy in clinical management of transplant candidates.

Histocompatibility in stem cell transplantation

P-757

CHIMERISM AFTER SYNGENEIC NON-CONDITIONED BONE MARROW TRANSPLANTATION IN BALB/C MICE

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Background: In advanced cell and gene therapies, the hematopoietic stem and progenitor cells are a favorable vector for transmission of beneficial and lasting therapeutic effect.
Aims: The main purpose of this study was to develop a mouse model, which would serve as a platform for future regenerative studies. We revisited the challenging topic of non-conditioned bone marrow transplantation (BMT), with the aim of achieving a therapeutically beneficial level of mixed chimerism in the syngeneic sex-mismatched non-conditioned mice.
Methods: 62×10^6 ($39–82 \times 10^6$) nucleated bone marrow cells were transplanted to 13 female BALB/c recipients and chimerism levels determined using contemporary qPCR technology, colony-forming unit (CFU) analysis, and FACS cell subpopulation sorting. Chimerism was determined in the bone marrow, blood, spleen, and lungs at 2, 6, and 12–17 weeks.
Results: At 12–17 weeks after the BMT, recipients' bone marrow contained on average $9.2 \pm 1.3\%$ of donor cells (n = 5). Spleen and lungs contained 2–3 times less donor traits, whereas blood matched the levels in the bone marrow. The percentage of donor hematopoietic CFU showed an average of $17.6\% \pm 3.7\%$ colonies of donor origin (n = 3). Purified neutrophils, B, and T cell exhibited $6.4\% \pm 1.2\%$, $6.8\% \pm 1.3\%$, and $2.9\% \pm 0.9\%$ chimerism (n = 3), respectively.
Summary/Conclusions: BMT led to successful engraftment of stem and progenitor cells and their subsequent proliferation. We confirmed the chimerism in the BM, on the level of committed progenitors, as well as in the purified myeloid and lymphoid cell lineages. The dose-response relationship for bone marrow was 0.13–0.22% engraftment per one million transplanted nucleated cells. This model of non-conditioned mouse BMT can be adopted as a method of choice in further hematopoietic regenerative studies.

Histocompatibility in organ transplantation

P-758

ABO INCOMPATIBLE KIDNEY TRANSPLANT WITH HLA CROSS-OVER RECOMBINATION: A RARE CHALLENGE

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Background: Renal transplantation is the best therapeutic option for some chronic renal terminal patients. Practiced at large scale for more than 50 years, strategies

have been developed in order to overcome the scarcity of available organs including use of brain-dead donors, donors in cardiorespiratory arrest and transplant from living donors. Pairing a donor-recipient with high probability of success for transplanted organ as well as for its receptor implies matching several systems. ABO blood group antigens, also present in vascular endothelial cells, cells of the distal convoluted and collecting tubules, are one of the most important factors of incompatibility in match selection. ABO natural antibodies may result in hyperacute graft rejection in minutes to hours. Similarly, antibodies against Major Histocompatibility Complex, or Human Leucocyte Antigen (HLA), or against endothelial antigens may, by activation of the complement system, lead to a hyperacute, acute or chronic graft rejection.

Aims: This case presents a patient with a previous kidney transplant from his mother, and a crossing-over recombination from his mother meiosis. He is candidate for an ABO incompatible kidney transplant from a sister presenting several HLA mismatches.

Methods: Patient and donors typed for ABO group system. Patient isoagglutinin titrations performed against commercial/donor cells. HLA genotyping done with sequence-specific primers. Patient anti-HLA antibodies screened and identified by Luminex system.

Results: Male patient, 39 years-old, group O positive, with mesangioproliferative glomerulonephritis due to IgA nephropathy, with a kidney transplant in 2004 due to chronic renal failure with live donor (mother), O positive. Patient HLA genotype: A*25,*29;B*08*44;C*07,*16;DRB1*03; patient's mother genotype: A*01,*25;B*08, *18;C*07,*12;DRB1*01,*03. An older sister was also genotyped: A*03,*25;B*18, *35;C*04,*12;DRB1*01,*13. After first transplantation, patient was immunosuppressed with daclizumab, cyclosporin, mofetil mycophenolate and prednisolone, and was regularly reassessed for screening and identification of anti-HLA antibodies (Luminex system) always with negative results. Chronic graft rejection evolved with progressive worsening of renal function but with no evidence of anti-HLA antibodies. Patient was proposed for a new kidney transplant (March 2017) and the only living donor available was another sister, age 38, blood group A positive. At pre-transplantation evaluation, patient had an anti-A titre of 1:64 IgG (commercial cells) and 1:128 (donor cells), and IgM 1:16 (both cells). According to the incompatible ABO transplantation protocol, patient isoagglutinin titres were not an exclusion criteria. Patient sister HLA genotype: A*03,*25;B*18,*35;C*04,*12;DRB1*01,*13. Several HLA incompatibilities were found with repeated incompatibilities from first donor (one HLA-A mismatch, and two in HLA-B, -C and -DR).

Summary/Conclusions: HLA genes have such a genetic proximity that they are usually inherited together according to a Mendelian model of inheritance for haplotype, where the phenomenon of gene recombination is relatively rare. Comparing haplotypes between mother, sisters and patient, an unequal crossing-over in meiosis of maternal origin for the patient between HLA-A and HLA-B loci was identified. Possibly due to an adequate immunosuppressant therapy after first transplantation (theoretically haplo-identical), evidence of anti-HLA alloimmunization was not demonstrated. However, this case today represents a challenge in transplantation by the several system incompatibilities either in ABO either in HLA.

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JOINT USE OF GSSP AND HIGH-RESOLUTION PCR-SSPS CAN RESOLVE GENOTYPING AMBIGUITIES OF HLA -A, B AND DRB1 BY SEQUENCE-BASED TYPING METHOD

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Background: The human leukocyte antigen (HLA) class I and class II loci are the most polymorphic genes in the human genome. Sequence-based typing has provided high-resolution typing of HLA, but genotyping ambiguity remains an issue.

Aims: To evaluate GSSP and high-resolution PCR-SSPs method for resolving ambiguous results from h HLA -A, B and DRB1 genotyping by sequence-based typing method.

Methods: HLA-A, B and DRB1 loci of all donors were genotyped by PCR-SBT. The genotyping ambiguities were resolved by joint use of high-resolution polymerase chain reaction- sequence-specific primers (PCR-SSP) and Group specific sequencing primer (GSSP) methods.

Results: 87.37% HLA-A, 93.54%HLA-B and 60.49% HLA-DRB1 ambiguities could be solved by GSSP method, 12.63% HLA-A, 4.76% HLA-B and 15.29% HLA-DRB1 ambiguities by high-resolution PCR-SSP method, and the rest 1.70% HLA-B and 24.22% HLA-DRB1 ambiguities by both GSSP and high-resolution PCR-SSPs method.

Summary/Conclusions: GSSP and high-resolution PCR-SSPs methods have high abilities to solve HLA -A, B and DRB1 ambiguities both locate inside and outside the sequencing region, respectively. GSSP and high-resolution PCR-SSPs methods can complement each other so joint use of GSSP and high-resolution PCR-SSPs can solve the problem of ambiguities in high resolution HLA typing.